VITAL FACTORS OF FOODS
VITAMINS AND NUTRITION

BY

CARLETON ELLIS, S.B.; F.C.S.

CONSULTING CHEMIST

AUTHOR OF "HYDROGENATION OF OILS, CATALYZERS AND CATALYSIS, AND THE GENERATION OF HYDROGEN AND OXYGEN," CO-AUTHOR "GASOLINE AND OTHER MOTOR FUELS," ULTRAVIOLET LIGHT, ITS APPLICATIONS IN CHEMICAL ARTS"

AND

ANNIE LOUISE MACLEOD, PH.D.

ASSOCIATE PROFESSOR OF CHEMISTRY, VASSAR COLLEGE

ILLUSTRATED

NEW YORK
D. VAN NOSTRAND COMPANY
EIGHT WARREN STREET
1922

PRINTED IN THE UNITED STATES OF AMERICA

"All one's work might have been better done; but this is the sort of reflection a worker must put aside courageously, if he doesn't mean every one of his conceptions to remain forever a private vision, an 'evanescent reverie'." — Joseph Conrad in *Notes on My Books.*

PREFACE

Investigations made during the past decade have brought forth a discovery of the utmost importance to the well-being of mankind. And the discovery is this: In the foods we eat or should eat there are present very small amounts of certain life-giving and disease-preventing bodies, the consumption of which is essential to growth and good health. Such bodies are called vitamins.

Before the day when the import of vitamins came to be recognized we were well satisfied to believe that an adequate supply of protein, fat, carbohydrate, mineral salts and water met our food requirements fully. Food charts were widely disseminated which made it apparent that the heat units of food, the " calories " available, were the essential factors in the control of a diet or ration. Women's clubs and other organizations were addressed on this score so often and emphatically that many a conscientious housewife kept scales in the kitchen to weigh protein, fat and carbohydrates and thereby compute the daily distribution and consumption of calories.

The common belief of past years, when the calorie or energy value of a diet was the theme, has since been modified. Fuel values are as important of consideration at the present time as they were in earlier years, but it is now well established that in addition to the body-building and fuel components of foodstuffs, namely, the protein, fat and carbohydrates and also salts and water, we should consume the accessory food substances or factors known as vitamins. That substances playing so vital a rôle in nutrition should until a decade ago have escaped discovery is surprising, so much so, in fact, that much incredulity was provoked over the announcement. The brilliant but necessarily laborious researches of investigators in this country supported by original as well as confirmatory work in England and elsewhere have largely overcome this scepticism, much of which apparently emanated from Germany, and have served to make the decade a monumental one in the dietary field. These revelations have made the question of *what to eat* an even more necessary matter of " food for thought." With foodstuffs constantly increasing in cost many are disposed to replace one food by another cheaper one on a calorie basis. This cannot be done with impunity. An

understanding of the vitamin requirements of the human organism and of the occurrence of vitamins in edible products as well as the effect of cooking operations on such food factors is needful.

The pronounced differences existing in various proteins due to their varying content of amino acids is no less a vital consideration. The effects of amino acids and the potency of vitamins has become the subject of constant research by a rapidly expanding group of investigators and information is accumulating at a surprising rate concerning the evaluation of foods from new vantage points.

This volume is offered in the endeavor to supply such information in a readily accessible form. It aims to furnish all essential facts regarding vitamins and to bring together the literature on the subject, thus to supply not only a textbook, but also a reference volume of a thoroughly comprehensive nature on these special but highly important phases of the nutrition problem.

The usage of the term vitamin seemingly needs no apology. Criticisms directed towards those workers in the field who have elaborated its fundamentals have made writers cautious in regard to terminology. At present there is little or no knowledge of the actual chemical nature of vitamins but the study of their functions has made most convincing progress. Owing to this feeling of scientific caution, vitamins often have been referred to in very general terms such as "accessory food factors" and their specific forms as fat-soluble *A*, water-soluble *B* and the like.

In the light of our present knowledge of vitamins and amino acids, and the noteworthy advances occurring in this domain, the supporters of vegetarianism possibly may find cause for reflection over some of their more extreme theories. A lowering of body tone and subsequent occurrence of symptoms of grave disease may be caused by a lack of vitamins in a diet employed over a series of years. The effects are in a way cumulative. The value of the all-vegetable over the normal diet requires contemplation anew from the vitamin standpoint. In the following pages these and many other features are discussed briefly or expansively according as our present knowledge enables their consideration.

Physicians who have been charging many of our ills to presence of toxins may have to retract the charges in some cases. It is becoming known that a number of maladies are caused, not by the presence of toxins but by the absence of vitamins. Such knowledge of "deficiency diseases" may lead to substantial changes in medical practice.

The authors have endeavored to make certain portions of the text

of a character which would likewise furnish the general reader with a knowledge of the practical side of vitamins including the different requirements by children and adults of these food factors, and if the volume be commended for simplicity of exposition as well as comprehensiveness, the authors will be gratified.

Our thanks are due to the Connecticut Agricultural Experiment Station, the Journal of Biological Chemistry, Professor E. V. McCollum, Professor J. F. McClendon, Dr. A. D. Emmett and associates, Professor H. Steenbock and associates for permission to use illustrations and charts relating to investigations on vitamins, and to Mr. B. Dass for various suggestions and for assistance in the reading of proof.

Ellis Laboratories,
92 Greenwood Ave., Montclair, N. J. — Carleton Ellis

Vassar College, Poughkeepsie, N. Y. — Annie Louise Macleod

INTRODUCTION

ELEMENTARY PRINCIPLES OF NUTRITION

The analogy between the animal body and a machine has been pointed out so often that it is almost a platitude, nevertheless it is particularly apt. Like a machine, the body must be provided with fuel, which it converts into heat and mechanical energy. Like a machine, the efficiency of the body depends largely upon the character and quantity of the fuel supplied. If the fuel is scanty there is loss of power; if it is piled on recklessly the system becomes choked with unburned material and waste products. As with machines, there is a maximum efficiency for each individual which can be secured only by careful and intelligent management, but too often, for want of management, the body, like the machine, is running below par.

For the nourishment of an animal there are six different classes of materials required, which are frequently spoken of as foodstuffs. These are fats, carbohydrates, proteins, water, mineral salts, and vitamins; the first three making up what are known as the organic foodstuffs. The great variety of edible materials are usually mixtures of these, generally containing all six in varying proportions. Each foodstuff has its special function in nutrition, and although the relative amounts of each can be varied very considerably without ill-effect, a well-balanced diet must contain all six.

Water, while not a food in the sense of being a source of nutriment in itself, is nevertheless most essential to the organism, as is proved by the fact that most starving animals die much more quickly if deprived of water than when water is obtainable. Water is required to dissolve the solid foods so that they can be absorbed by the tissues and carried from place to place by the blood, and also to dissolve and carry off waste products from the tissues. Since water is continually being eliminated from the body in the urine and the perspiration it is necessary to replace the loss. Many of our foods, especially fruits and vegetables, contain a large proportion of water, but in the ordinary diet additional amounts must be obtained by drinking water. Dr. Hawk and his collaborators at Jefferson Medical College have

conducted a long series of experiments from which it would appear that copious water-drinking to the extent of a quart or more with each meal has many beneficial features. In one subject such procedure resulted in a gain of two pounds in five days.

The minerals of the diet are necessary for the building and repair of the bony structure of the body, and also serve in less obvious ways to maintain the proper concentration of the body fluids and the tone of the cells. Moreover, certain mineral elements enter into the composition of the body substance, as iron, which forms a part of the haemoglobin of the blood, and others have specific rôles, like calcium which plays an important part in regulating the beating of the heart, and sodium which exercises a profound influence on the irritability of the muscles. Fruits, vegetables, and milk are all excellent sources of mineral matter in the diet.

The organic foodstuffs, fats, carbohydrates, and proteins, are all complex chemical compounds which must be broken down by the process of digestion before they can be utilized by the system.

Fats are broken down in the intestine into fatty acids and glycerine, which are absorbed through the intestinal wall and apparently recombined into the particular body fat characteristic of the species of animal utilizing it. This fat can be stored up in the tissues until needed by the organism as a source of energy.

Carbohydrates include sugar, starch, and allied compounds. They also must be broken down by digestion into simple compounds before absorption can take place, and these digestion products may be used at once as a source of energy, or built up into a new form of carbohydrate for storage, or converted into fat and stored in the tissues in that form.

Proteins differ from the other foodstuffs in containing nitrogen in the only form in which this element can be utilized by the animal body. The most diverse substances are protein in nature. The muscle tissues of all animals, white of egg, and the curd of milk are the principal animal proteins used as food. All vegetables and fruits contain more or less protein, but peas, beans, and nuts are the chief vegetable sources. Inasmuch as nitrogen is an essential part of animal tissues and the animal can obtain the necessary nitrogen only in the form of protein it is obvious that this class of foodstuffs is of peculiar importance. The relative proportions of fat and carbohydrate in the diet can be varied almost at will, since either one can be utilized as a source of energy or converted into reserve fat, but there is a minimum amount of protein necessary for every organism without which the necessary nitrogen for tissue building or repair will

be lacking. If excess of protein above the requirement is eaten a small portion may be converted into fat, but most of it is decomposed and excreted as waste.

Proteins are combinations of smaller units known as amino-acids. Mankind requires a certain amount of specific amino acids for proper nourishment. The various proteins have been found to possess different biological values according to their power to supply these constructive food elements essential to the maintenance of the body in proper nitrogenous equilibrium. There are eighteen principal amino acids, and a few more of lesser importance. The principal amino acids make a formidable list. These are glycine, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, serine, cystine, aspartic acid, glutamic acid, ornithine, arginine, lysine, histidine, proline, oxyproline, tryptophane.

Amino acids are combined in different proportions to form the enormous variety of proteins, somewhat as the twenty-six letters of the alphabet are combined into an almost unlimited number of words. The specific character of the individual proteins is due to the relative proportions in which the amino acids are present. In the course of digestion the proteins are decomposed into amino acids, are absorbed in that form and are carried by the blood to the various tissues, which select what they need for building and repair. The residue is then carried to the liver, decomposed there, and the waste excreted.

Careful observations have shown that the nutritive value of a protein depends on its content of amino acids, and since this has become established, enormous strides have been made in the science of nutrition, for it is possible to find out by experiment which proteins provide the best combination of amino acids for a given species of animals and how to supplement one protein which is poor in a certain amino acid by another in which it is present in excess. (See Chap. III.)

The energy of the body is supplied by the slow burning (oxidation) of the food. If the food supply is insufficient, the tissues will be used up instead. This energy appears partly as muscular force, and partly as heat. The more work done by the muscles, the more energy is required and the more food must be burned. It has been found that the energy set free when food is burned inside the body is exactly the same as that set free when it is burned outside the body, and consequently the energy-value of a food can be measured by burning it in a special form of apparatus, the calorimeter. The unit of energy used in nutrition is the calorie, and is the amount of heat which would be required to heat one pound of water about 4° F., or the equivalent of this. The calorific value of a given amount of a particular food

is the number of units of energy which it will set free when it is burned.

It is also possible to measure the energy used up in performing any kind of muscular work and to express this in calories. The relation between the energy output of the body and the available energy from the food eaten can therefore be readily calculated. Clearly, if an animal performs a piece of work which requires one hundred calories

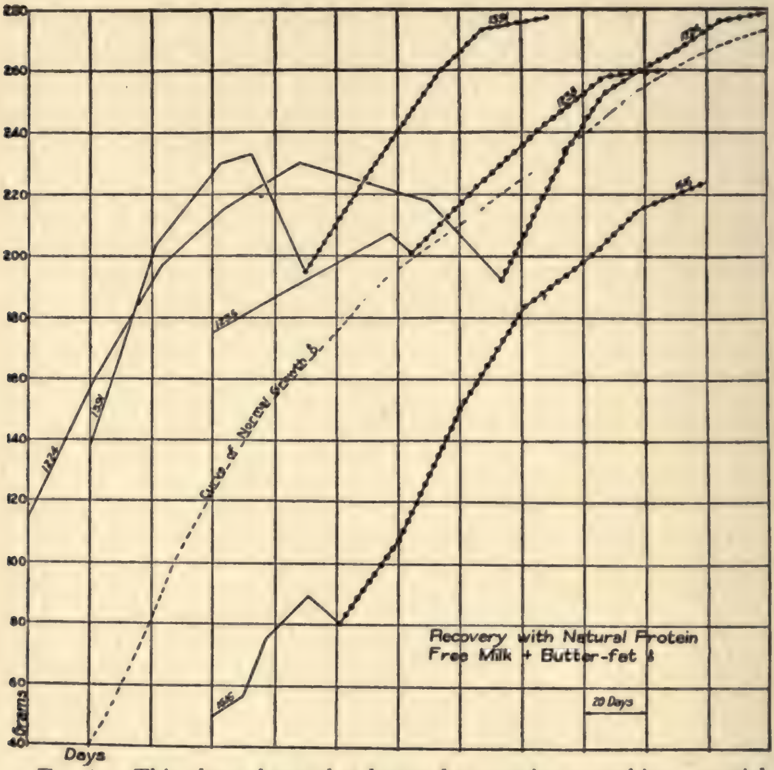

FIG. 1.—This chart shows that butter-fat contains something essential for normal growth. The curves indicate that after feeding a diet of purified foodstuffs, the fat being lard, the animals after growing normally for several weeks suddenly began to lose weight. When a part of the lard was replaced by butter-fat (shown by the beaded line), they immediately recovered. These animals would have died in a few days if this change had not been made.

Courtesy of the Connecticut Agricultural Experiment Station (Bulletin 215).

of energy it will require to consume an amount of food which will supply one hundred calories or else it must use up a portion of its

own tissues to make good the deficit. Even when no voluntary work is being done and the body is resting as completely as possible, energy is being used in the beating of the heart, the movements of the stomach and intestines, the rise and fall of the chest in respirations, and so on. This irreducible minimum of energy is called the basal metabolism of the individual and varies somewhat with the body weight, surface, age and sex. A vast number of measurements have been made on human beings of various ages and averages for different types have been ascertained, which may safely be taken as close enough to the actual basal metabolism of any given individual. By keeping account of the time spent in various activities in the course of twenty-four hours, calculating the energy required for each, and adding the values thus obtained to the basal metabolism, a measure of the daily energy requirement of the individual can be found. From this observation the amount of food required to supply that energy can be determined. A slight excess over and above this demand will be required by adults to provide for the repair of the tissue, and a larger allowance is needful for the growth of the young.

CONTENTS

INTRODUCTION. ELEMENTARY PRINCIPLES OF NUTRITION.................. ix
The foodstuffs — Water — Minerals — Fats — Carbohydrates — Proteins — Amino-acids — The energy value of food.

CHAPTER I. VITAMINS, — WHAT THEY ARE AND WHAT THEY DO........ 1
Food in terms of calories — Unanswered problems — Early experiments with synthetic diets — A new dietary essential — "Vitamins" — Nomenclature — Why the vitamins were so long over-looked — How the vitamins act — Historical account of the development of the science of nutrition and the vitamin hypothesis.

CHAPTER II. EXPERIMENTAL METHODS.................................. 18
Types of animals employed — Comparability of requirements of different species — Technique employed with different types of animals — General considerations — Necessity for long-continued experiments — Variability of experimental animals — Misleading evidence — Preventive as compared with curative experiments — Food intake — Technique of yeast tests for vitamin B — Standardization of methods.

CHAPTER III. DIETARY FACTORS OTHER THAN VITAMINS 46
McCollum's "biological analysis" of foods — Dietary efficiency of various grains, seeds, leaves, and roots — Relative nutritive value of various proteins — Basal diets — Mineral salts.

CHAPTER IV. DISTRIBUTION OF THE VITAMINS......................... 66
Vitamin A in animal fats — Comparative nutritive value of lard and lard substitutes — A in vegetable fats — In leafy vegetables and grasses — In tubers and roots — In fruits — In seeds — Factors influencing the value of milk and butter as sources of A — Correlations between content of A and function — And pigmentation — Vitamin B in yeast — In seeds — Localization within the seed — Correlation between content of B and phosphoric anhydride in cereals — Green's "vitamin index" — B in grasses — In common vegetables — In fruits — In animal products — Vitamin C in fruits — In dry seeds — In germinated seeds — In common vegetables — In milk — In eggs — In meat.

CHAPTER V. STABILITY OF THE VITAMINS WITH REGARD TO CONDITIONS OF HEATING, DRYING, AND STORING.................................. 89
Conflicting evidence — Stability of A as found in animal fats — Effect of aeration — Of ultraviolet light — Stability in animal tissues — In vegetable tissues — Does A survive drying and canning? — Stability of B in seeds and grains — In yeast — In common vegetables — In animal products — In milk — In canned and sterilized foods — Effect of radium emanation — Sensitiveness of C to heat and desiccation in general — Does presence of acid increase stability? — Variation in stability in different vegetables and fruits — Effect of aeration during heating Antiscorbutic power of heated milk — Effect of drying and storage on content of C in vegetables and fruits.

Chapter VI. Chemical Structure and Properties of the Vitamins. 111
Vitamin A — Solubilities — Action of alkalies — Destruction during hydrogenation of fats — Phosphorus and nitrogen content — Vitamin B — Solubilities — Adsorption — Stability towards acids and alkalies — Isolation and purification — Theories as to chemical structure — Properties of active substances isolated — Vitamin C — Solubilities and adsorption — Effect of acids and alkalies on thermostability — Qualitative test for presence of C.

Chapter VII. Concentrated Preparations of Vitamins.............. 136
Concentrates of A — Dried plant products as source of A — Concentrates of B — Preparation by evaporation of food extracts — By precipitation — By "activation" of adsorptive medium — Miscellaneous methods of preparation — Concentrates of C — Preparation by evaporation of extracts — Commercial vitamin preparations.

Chapter VIII. How many Vitamins are there?..................... 150
Are the growth-promoting and antineuritic properties of vitamin B due to two distinct factors? — Experimental evidence — Comparative data concerning distribution, minimal requirements, behaviour towards precipitants, solubilities, stability — Miscellaneous experiments.

Chapter IX. Rôle in Nutrition.................................. 162
Requirements for maintenance and growth — Storage of vitamins in the tissue — Comparative requirements of different species — The antiscorbutic requirements of the rat — Apparent synthesis of vitamins by animals on vitamin-free diet — Symptoms due to deficiency of A — Of B — Of C — Relation of vitamins to calcium metabolism — Tooth decay caused by deficiency — Connection with carbohydrate metabolism — Nitrogen metabolism — Physiological function as indicated by distribution in tissues — Relation of vitamins to the nerve tissue — To the glandular organs — Vitamins as hormones.

Chapter X. Rickets.. 190
Symptoms — Macroscopic and microscopic character of rachitic tissues — Incidence — Theories of origin — Relation to fat deficiency — Effect of hygienic conditions — Rickets and diet — Mellanby's theory of antirachitic factor — Other theories.

Chapter XI. Beriberi.. 201
Incidence — Early theories of origin — Experiments in Japanese navy — Polyneuritis gallinarum — Dietetic origin — Symptoms — Susceptibility of different species — Infection theory — Intoxication theory — "Polished rice" — Nutritive deficiencies of polished rice — Vitamins and beriberi — Antineuritic foods and curative agents.

Chapter XII. Scurvy... 217
Historical — Symptoms — Barlow's disease — Guinea pig scurvy — Early theories of origin — Chronic poisoning — Bacterial infection — Constipation — Deranged calcium metabolism — Vitamin hypothesis — Antiscorbutic foods.

Chapter XIII. Xerophthalmia..................................... 234
Symptoms as observed in experimental animals — In children — Growth on a fat-free diet — Relation to deficiency of A — Infection theory — Effect of unbalanced diet.

Chapter XIV. Pellagra... 242
Historical — Incidence — Symptoms — Theories of origin — Infection

— Relation to sanitary conditions — Dietary deficiency — Goldberger's work — Animal experimentation — Curative diets — Treatment by vitamin preparations — The protein of the diet in relation to pellagra.

Chapter XV. Milk.......... 261
Constituents of milk — Variation in milk of different species — Fat — Lactose — Proteins — Nutritive value of milk proteins — Inorganic elements — Vitamins — Variation in milk due to variation in diet of lactating animal — Amount — Fat content — Protein — Lactose — Mineral content — Vitamin content — Seasonal variation — Effect of heat on milk — Pasteurization and sterilization — Nutritive value of boiled milk — Value of heated casein — Calcium content of heated milk — Effect of heating on vitamins — Antiscorbutic value of pasteurized milk — Evaporated and condensed milks — Dried milk — Nutritive value — Vitamin content.

Chapter XVI. Vitamins for the Baby.......... 292
Effect of vitamin deficiency in early life — Breast feeding — Importance of diet of nursing mother — Cow's milk as food for the baby — Raw and heated milk — Enriched milks — Beneficial effect of added B — Vitamin content of "modified milk" — Antiscorbutics for infants — Vitamin reserve.

Chapter XVII. Old Foods through New Eyes. What to Eat from the Vitamin Standpoint.......... 300
Vitamin requirements — Animal foods as source of vitamins — Meat — Fish — Eggs — Milk — Butter — Oleomargarines — Cheese — Animal fats — Vegetable foods — Seeds — Cereals — Grains — Tubers and roots — Leafy vegetables — Fruits — Nuts — Vegetable fats — Yeast — Cooked foods — Canned and preserved foods — Dried foods — Balancing the diet with respect to vitamins.

Chapter XVIII. The use of the Vitamins in Clinical Medicine..... 309
"Deficiency diseases" the least important consequence of vitamin deficiency — Relation between vitamin deficiency and susceptibility to infection — Therapeutic value of yeast — Beneficial effects of vitamin administration in cases of malnutrition.

Chapter XIX. Vitamins and the Lower Organisms.......... 313
The "Bios" of Wildier — Stimulation of yeast growth by vitamin — Vitamin requirements of fungi and moulds — Of bacteria.

Appendix. Tables of the Distribution of Vitamins.......... 320

VITAL FACTORS OF FOODS

CHAPTER I

VITAMINS,—WHAT THEY ARE AND WHAT THEY DO

The problem of adequate and economical nutrition — admittedly a problem of primary importance to mankind in general — may perhaps be said to have developed into an experimental science at the beginning of the 17th century, when **Sanctorius**[1] endeavored to keep himself at normal weight by having a huge pair of scales made with a chair suspended from one arm and counterpoised by the opposite scale pan. Distrusting the guidance of instinct or appetite, it was his custom to arrange a definite weight on the pan each day, seat himself in the chair and eat until the scale dipped. Although history relates little as to the progress of this experiment it is safe to conclude that the philosopher had some surprises in the course of it, and it is not strange that he did not advance very far towards a solution of the fundamental question of nutritional economy. Sanctorius died, and was forgotten or only remembered with a smile, but a later and wiser generation fell into a somewhat similar error.

Following the discovery of the relation between the quantity of heat given off when an organic food stuff was burned in a calorimeter and the energy furnished by the slow oxidation of that same food stuff in the animal organism, came the development of the calorie theory. It seemed so logical and so simple (once the mechanical difficulties had been surmounted) to calculate the amount of energy used up by a living animal in the course of twenty-four hours, and then to provide an amount of food which would furnish just that much energy, due allowance being made for unavoidable waste through lack of assimilation, for the essential protein for repair of the tissues, and for growth in the case of the young. Surely the height of efficiency was within reach. The provisioning of a family became a matter of simple arithmetic, so many ounces, so many calories. The calorie was a popular catchword, and the conscientious housekeeper weighed and measured and counted calories till her brow

[1] **De medicina statica aphoresmi, Venice, 1614, Translated by John Quincy, M. D., London, 1737.**

was furrowed with care, wondering vainly meanwhile where the energy went to when John ate 1500 calories more than the most generous accounting could possibly allow him. Battles raged round the calorie. Should we try to "train down" to our rightful allowance or should we forget calories so far as our purses would allow us? What was the irreducible minimum in calories? How many of them must come from protein? How many might come from protein? Did it make any difference whether it was animal or vegetable protein and why? Bold spirits asserted firmly that we should follow our "God-given instincts," and pointed to many a hale and hearty *bon-vivant* of ripe age. Instances were multiplied of people who were saved from untold miseries by decreasing their calories, by increasing their calories, by a high protein diet, by a low protein diet. The observant advertiser took up the cry and our attention was called to the fact that for an insignificant sum we could buy sufficient calories of cereal food to maintain us for a day, a week, or a month, while the same sum expended for the more appetizing steak would hardly provide sufficient energy for an hour.

But logical as this reasoning was, convincing as were the indisputable figures brought as proof, feeding by calories alone proved unsatisfactory. The first and most obvious cause of this lay in the qualitative differences which were shown to exist between different proteins. Proteins are composed of the units known as amino acids, but they differ in the proportions of the various amino acids present in the combination. Certain of these acids are more essential for the living animal than are the others, and if a given protein is poor in these essentials it will prove an unsatisfactory food as compared with others in which they are more abundant. In the second place, it was found that certain foods, although they contained all the necessary organic constituents and provided more than sufficient calories nevertheless failed to nourish an animal because they did not contain the inorganic salts required by the system. Careful experimentation showed how this lack could be remedied, but still the problem of nutrition was not solved.

Beginning with **Lunin** in 1881 various investigators endeavored to keep experimental animals alive on "synthetic diets," combinations of pure food substances put together in the proper proportions to reproduce as far as possible natural foods which were known to be adequate. One after the other reported failure. **Lunin** found that while adult mice could be kept alive for several months on a diet of milk alone they invariably died in less than one month if restricted

to a mixture of water, milk protein, milk fat, milk sugar, and the ash of milk in which all its inorganic constituents were present, from which he concluded that there must be some indispensable substance in milk apart from the ordinarily recognized constituents. Other investigators made similar discoveries. In England **Hopkins** fed young rats on a mixture of purified foodstuffs and found that if the purification was carried out with sufficient care the rats failed to grow, but that the addition of an extraordinarily small amount of milk apparently provided the missing ingredient and normal growth took place. In America, **Osborne** and **Mendel** observed that young rats fed on a diet of protein, starch, lard, and a solution containing the mineral salts of milk would grow for a short time but invariably began to fail before reaching normal size. So slight a change as the substitution of butter for lard caused resumption of normal growth and the health of the animal improved. **McCollum** and **Davis** found that the missing food substance could be obtained from the ether extract of eggs or butter, whereas lard and olive oil, although so similar in chemical nature to the latter, were valueless in this respect.

Meantime **Funk** had been studying the disease known as beriberi. The symptoms characteristic of this disease could be produced in pigeons by feeding them on a diet of polished rice, and Funk found that birds which were at the point of death from this cause were cured with astonishing rapidity by a little of the alcoholic extract of the rice hulls. **Funk** assumed that the disease was due to the absence of some essential constituent which was present in the outer layer of the rice grain and was removed in the process of milling. To this substance, the nature of which was unknown, he gave the name *vitamine,* a name which has been severely criticized as misleading, but which has nevertheless found a permanent place in the literature. The general resemblance betwen the substance of which a small amount prevented or cured beriberi and the substance of which a small amount seemed necessary to promote growth, led to the term *vitamine* being used for both, and finally this term came to signify a certain class of unidentified factors present in very small amounts in natural foods but essential for growth and well being of the animal organism.

Since the term *amine* has a certain definite significance in organic chemistry, which is not intended to apply in *vitamine,* **Hopkins** has suggested the term "accessory food substances" instead, a usage which is followed by many English writers. **McCollum** objects to this terminology however on the ground that "accessory" has a connota-

tion of "dispensable" while the substances referred to are essentials, and made the suggestion that the growth-promoting substance present in butter-fat should be designated "fat-soluble *A*," and the anti-beriberi substance "water-soluble *B*" because of their respective solubilities in fats and in water respectively. A new term "advitants" has recently been introduced as a substitute for "vitamines," but it remains to be seen whether or not it will find general acceptance.[2] In the present work the nomenclature adopted is that suggested by **Drummond**,[3] according to which the final "e" of vitamine is dropped,[3a] thus avoiding the objectionable "amine" termination, while retaining a name that has become familiar through long usage.[3b] The different classes of vitamins are distinguished as *A*, *B*, and *C*.

A third member has been added to the vitamin class, the "*C*" or scurvy-preventing vitamin, which is the substance long known to be present in lemon-juice and which acts as a preventive or cure for scurvy. Whether or not we shall ultimately recognize the existence of still other vitamins cannot at present be confidently predicted. There is already certain evidence which would seem to indicate the possibility of two distinct substances at present classed together as "vitamin *B*," one of which functions as a beriberi preventive while the other is of importance in growth, but so far the evidence is inconclusive. This question will however be dealt with in more detail in a later chapter.

It may be asked, how can the existence of a whole class of substances of fundamental importance in the nutrition not only of man but of all animals have so long escaped observation? The explanation is based partly on the wide distribution of the vitamins in natural foods and partly on the minute quantities required to maintain an animal in at least comparatively good health. Under ordinary conditions, even on what would be considered a most restricted diet, traces of vitamins are always present and these traces are sufficient to prevent obvious disease, although it is probable that many cases of abnormality, debility, and general lack of tone, if not actual disease, will be found attributable to lack of sufficient vitamins in the diet. It is only when a food has been so treated as to remove or destroy

[2] J. Chem. Soc. 1920, 117, 1201.

[3] **Drummond**, Bioch. J. 14,660, 1920.

[3a] **Funk** does not endorse Drummond's proposal to drop the final "e" in the term "vitamine." (J. Ind. Eng. Chem. 1921, 1110.)

[3b] The word vitamin has been pronounced in a variety of ways, the approved pronunciation being vy'-ta-meen.

the vitamins naturally present that the results become so evident as to force themselves upon the attention. By an unfortunate perversity the present day manufacturer not infrequently endeavors to make his products more attractive by processes which seriously reduce the vitamin content, and hence the nutritive value, as in the treatment of cereals when the vitamin-rich germ and cortex are removed, or in the overheating of foods when drying for the purpose of preservation. A striking illustration of the result of this is seen among the rice-eating people of the Orient where beriberi followed the introduction of modern milling machinery by which the germ and "silverskin" of the rice grain, and in these the whole amount of vitamin *B*, is removed. In the West the food is more varied, and it is unlikely that any diet would not contain a certain amount of all three vitamins; whether the amount is always adequate is however open to serious question.

A second reason why the vitamins escaped detection so long is because their absence is indicated only by a subnormal condition which may usually be ascribed to other causes. Ordinarily, lack of vitamin will be accompanied by other unfavorable conditions; moreover the morbid state of the organs which resulted from vitamin deficiency might easily be regarded as the cause, instead of merely one of the symptoms of malnutrition.

Finally, there is the wide-spread tendency to look for a *positive* rather than a *negative* cause of disease; for the presence of an actively injurious substance rather than the absence of an essential nutritious element. It was only when carefully conducted feeding experiments were carried out with healthy animals kept under ideal conditions in every respect save the one under investigation, that failure of health could be traced to its true source.

How the vitamins act, what part they play in the life process, we cannot say definitely. When we have described their general functions — growth-promoting, beriberi- and scurvy-preventing — and their distribution, and have emphasized their indispensability, we have said almost all that we know positively. Small, even minute amounts are surprisingly effective, a fact which suggests that they are of the nature of catalysts, agents which have the power of bringing about extensive chemical change without being themselves altered in the process. Practically all of the complex reactions in the living cell are believed to be brought about by the catalytic action of the enzymes present in the cell, and it would not be difficult to imagine an essential rôle for certain catalysts which do not exist

pre-formed in the organism but must be obtained from the food. On the other hand, the degenerative change in the vital organs, particularly the ductless glands, would suggest the possibility of an intimate connection between the vitamins and the internal secretions. They may be hormones, that is, stimulants in the absence of which the glands fail to produce the normal secretion, or possibly they may be essential though almost infinitesimal constituents of certain tissues, which cannot be synthesized in the animal body, and without which those tissues cannot develop.

Such evidence as we have on these points will be given in a later chapter.

Historical

As indicated in the foregoing, the discovery of vitamins as essentials in the diet of animals followed from attempts to maintain animals on restricted diets of synthetic food mixtures.

Lunin,[4] in 1881 compared the growth of mice fed on milk with those on a diet made up of milk sugar, milk fat, milk protein, and the mineral constituents of milk, and found that while adult mice could live in apparently good health for several months on the milk diet they invariably died within one month on the synthetic mixture. He draws the conclusion that "other substances indispensable for nutrition must be present in milk besides caseinogen, fat, lactose, and salts," but this sapient remark was practically ignored for over thirty years.

Henriques and **Hansen** [5] endeavored to maintain white rats on isolated proteins with the addition of sugar, lard, cellulose, and inorganic salts, and noted a gradual loss of body weight in each case.

Hopkins [6] studied the nutritive value of purified proteins supplemented by carbohydrates, fats, and mineral salts, and found that neither growth nor maintenance of weight could be secured on these diets. He writes in 1906 (*l. c.*):

> It is probable that our conception of stimulating substances may have to be extended. The original vague conception of such subjects being condiments, chiefly affecting taste, gained in definiteness by the work of the Pavlov School. But the place of specific diet-constituents which stimulate the gastric secretory mechanism can be taken by the products of digestion itself, and in this

[4] Ztschr. f. physiol. Ch. 1881, 5, 31.

[5] Ztschr. f. physiol. Ch. 1904–5, 43, 417, 1908, 54, **Henriques**, *Ibid.* 1909, 60, 105.

[6] **Willcock** and **Hopkins**, J. Physiol, 1906, 35, 88, **Hopkins**, Analyst, 1906, 31, 395.

connection the stimulant in the diet is by no means indispensable. Most observers agree that the addition to normal dietaries, of meat extracts capable of stimulating the gastric flow does not increase the actual absorption of food, though this point could be properly tested by adding them to artificial dietary known to be free from analogous substances. As was emphasized above, the milk did not affect absorption in my experiments. But such undoubted stimulating effects due to diet constituents as those discovered by **Pavlov** may quite possibly be paralleled elsewhere in the body on more specific and indispensable lines. Stimulations of the internal secretions of the thyroid and pituitary glands which are believed on very suggestive evidence to play an important part in growth processes, can be legitimately thought of. On the other hand, the influence upon growing tissues may be direct. If the attachment of such indispensable functions to specific accessory constituents of diets is foreign to current views upon nutrition, so also is the experimental fact that young animals may fail to grow when they are absorbing daily a sufficiency of formative material and energy for the purpose of growth.

Falta and **Noeggerath**[7] fed rats on various purified proteins, starch, sugar, and fat, with the addition of salts, water, and in some cases lecithin, cholesterol, and sodium nucleate, all obtained as pure as possible. In most cases there was a steady decline in weight throughout the experimental period, followed by death, although occasionally the body weight was maintained fairly well for the first few weeks. In no case did they succeed in keeping the animals alive more than 94 days, and usually death ensued in shorter time. On the other hand they were able to keep rats in health on milk alone for periods of six months or more.

Jacob[8] used pure starch, olive oil, casein, sugar, salts, and cellulose as a diet for pigeons and rats, and concluded that the lack of success in this and similar experiments was partly due to the unsatisfactory physical character of the diet and, probably, partly to the difficulty of inducing the animals to eat sufficient of a food which was unpalatable. **McCollum**[9] noted the same difficulty in feeding rats on complex artificial mixtures of purified foodstuffs, and attempted to overcome it by frequent changes in the combinations of foodstuffs used and by addition of flavors. He concluded that palatability is a most important factor in animal nutrition, and that the failure of previous efforts to maintain animals on mixtures of isolated foodstuffs was due to lack of palatability. Although he was successful in keeping his animals alive for comparatively long periods they failed to maintain their weights. In this connection **Osborne** and **Mendel** have pointed out the difficulty of deciding whether the failing appetite invariably observed is a cause or result of imperfect nutrition.

[7] Hofmeister's Beitr. Z. Chem. Physiol. 1905, 7, 320.
[8] Ztschr. f. Biol. 1906, 48, 19. [9] Am. J. Physiol. 1909, 25, 120.

That a monotonous and unaccustomed food may be used successfully over long periods of time without ill-effects was proved by the experiments of **Falta** and **Noeggerath** already referred to, in which rats were maintained successfully for six months or more on monotonous diets of milk, milk powder or lean horsemeat; by **Socin**,[10] who kept mice in good health for 90 days on egg-yolk, starch, and cellulose, and by many other experimenters.[11]

In the light of our present knowledge many of these early experimental diets were obviously lacking in amino acid or mineral content or both, nor were the conditions ideal in other respects, nevertheless the multiplication of evidence seemed to point to an inexplicable difference between natural foods and the synthetic mixtures designed to be qualitatively and quantitatively equivalent to them. **Rohmann**[12] indeed claimed to have kept mice indefinitely and secured reproduction on food mixtures made up of purified components, but there is reason to believe that his success was due to the fact that the food substances used still contained the essential constituents in no inconsiderable amount.

In 1909 **Stepp**[13] began a series of experiments to determine whether animals are dependent on their food supply for lipoids or can furnish them by synthesis. He fed materials extracted with ether and alcohol to mice and noted that without exception the mice succumbed in a few weeks when fed otherwise adequate food mixtures that had been thoroughly extracted. When the extracted material was replaced the food was rendered sufficient for maintenance. The lacking substance was assumed not to be inorganic since the addition of ash from the lipoid extracts failed to maintain the mice. **Stepp** concluded that the essential factors were not fats, since addition to the extracted food of butter, tripalmitin, tristearin, triolein, lecithin, and cholesterol in turn was without effect. The missing substance could however be extracted from skimmed milk.

In 1911 **Osborne** and **Mendel**[14] published an account of an elaborate series of feeding experiments on rats, with isolated food substances, designed to compare the nutritive value of various proteins, using as a basal diet, starch, sugar, lard, agar (as roughage), and a mixture of inorganic salts. Among the many valuable points brought out in

[10] Ztschr. f. physiol. Ch. 1891, 1, 15, 93.

[11] A more complete account of the early nutrition experiments is given by **McCollum**, Am. J. Physiol. 25, 120.

[12] Alleg. Med. Cent. Zeit. 1903, No. 1, 1908, No. 9.

[13] Bioch. Ztschr. 1909, 22, 45a; Ztschr. f. Biol. 1912, 57, 135, 1912-13, 59, 366.

[14] Carnegie Pub. 156, *Pts.* 1 & 2.

their monograph was the fact that even when this diet was supplemented by a sufficient amount of protein and consumed in amounts which fulfilled the energy requirements of the animals it nevertheless invariably failed either to induce substantial growth in young rats or to satisfy completely the maintenance requirements of older ones over long periods of time. Having observed that rats could be grown from an early age and even resuscitated after nutritive decline

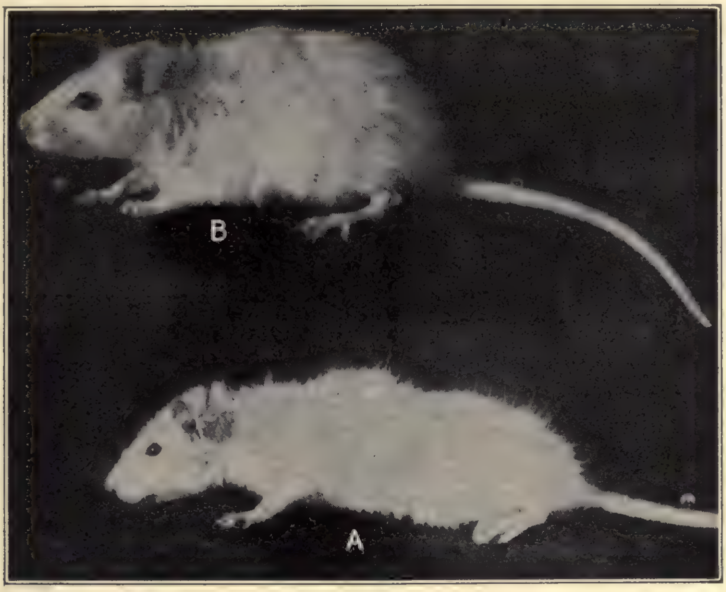

FIG. 2. — The picture (A) shows a rat which had been fed for one month on a diet deficient in water-soluble vitamin. At this time the animal was so weak it was scarcely able to stand and would have died in a few hours if some source of this vitamin had not been furnished. After the picture was taken a small daily dose of yeast which is rich in the water-soluble vitamin was given to the rat, the food remaining otherwise exactly as before. Twelve days later the picture (B) was taken. The result is apparent.

Courtesy of the Connecticut Agricultural Experiment Station (Bulletin 215).

by a diet of milk powder mixed with lard and starch, they were led to suspect that the essential factor or factors might be found in the protein-free portion of the milk, and accordingly milk which had been freed from fat, protein, and water, was used to replace the inorganic salt mixture and part of the carbohydrate in the basal ration, with results which were surprisingly successful. When 28.2%

of this "protein-free milk" powder was incorporated in the diet not only was satisfactory growth obtained in the case of young animals, but recovery was promptly manifested in practically every case of nutritive decline due to unsatisfactory food.

Somewhat similar results were obtained by **Hopkins** in continuance of his earlier work and published by him in 1912.[15] Using an artificial diet similar to that of **Osborne** and **Mendel**, consisting of casein, fat, starch, sugar, and inorganic salts, which were prepared by mixing equal parts of the ash of oats and dog biscuits, **Hopkins** added milk itself, but this was given in such small quantity that the total solids contained in it amounted to no more than from one to three or four per cent of the whole food eaten. This small addition induced normal growth upon dietaries which without it were incapable even of maintenance. In those experiments in which the basal diet contained fully purified material the rats without milk soon failed to grow. When the constituents were less completely purified, as when the protein was a commercial preparation of casein, slow growth occurred. In all cases the addition of small amounts of milk induced normal and continued growth. A similar, but less pronounced effect was obtained with the protein-free and salt-free extracts of milk solids and of yeast. The total energy consumption of the animals under comparison was carefully determined, and it could be shown that the rats upon the purer dietary ceased to grow at a time when their intake was more than sufficient quantitatively to maintain normal growth. If growth continued (as upon the less pure basal dietary) it could be shown that the small milk addendum reduced the food consumption necessary for given weight increment to one half or less. The milk ration was fed separately and in advance of the administration of the main dietary. It could not therefore affect the palatability of the food or diminish its monotony. In general, moreover, it was found that cessation of growth upon the pure dietary took place before any failure in appetite, although the consumption might later fall to a lower level. Any effect of the addendum upon the appetite must therefore have been secondary to a more direct effect upon growth processes.

In a later publication **Osborne** and **Mendel**[16] confirm their earlier results, but are still in doubt as to the cause. "Whether the deficiency of the purely artificial diet is to be attributed to improper proportions of its constituents, to improper combination of these constituents, or to the lack of some essential element, is at present difficult to define."

[15] **Hopkins**, J. Physiol. 1912, **44**, 425.

[16] J. Biol. Chem. **13**, 233, 1912–13.

They claimed however that preliminary experiments had indicated a considerable degree of success in nutrition in the absence of the hypothetical "hormone" which was assumed to be present in the milk. Rats were said to have been maintained in good health for as long as 400 days on a diet composed of a purified protein, starch, sucrose, and a salt mixture of inorganic salts in the proportions in which they are found in milk.

This was disputed by **Hopkins** and **Neville**[17] who reported that when rats were fed on the experimental ration of **Osborne** and **Mendel,** composed of very carefully purified substances they were unable to obtain any appreciable growth, and the animals, in spite of a satisfactory consumption of food, were all dead before the expiration of 40 days. When as little as 2 cc. of milk per day was given in advance of feeding with the **Osborne** and **Mendel** diet, steady growth resulted, although distinctly slower than in the earlier experiments of **Hopkins.**

Osborne and **Mendel**[18] subsequently admitted that the milk-free diet was less satisfactory than they had at first believed. "It is true that in several instances we have succeeded in keeping grown rats in health and in apparent nutritive equilibrium on purely artificial food mixtures over periods far longer than the experience of our predecessors had led us to expect. But the outcome has never been satisfactory in the sense of extending over what may be considered as the larger portion of the life span of an adult animal." Rats fed on purified protein, lard, starch, and protein-free milk grew excellently for sixty to a hundred days, but sooner or later thereafter normal growth stopped. The animals remained at constant weight for a few days, or grew very slowly, and then suddenly declined and died unless a change was made in the diet. They prepared various mixtures of salts designed to imitate as closely as possible those salts present in milk, and found that while under certain conditions there was very considerable growth, nevertheless in most cases this ceased sooner than that induced by the natural protein-free milk.

The manifest superiority of natural milk foods over either "natural" or "artificial protein-free milk," which had been demonstrated by **Hopkins,** having been thus confirmed by **Osborne** and **Mendel,** these investigators arrived at the conclusion that the essential factor must be associated with the cream which had been removed in the preparation of the "protein-free milk." When the lard which they had used as the fat component of the diet was replaced by butter-fat the result-

[17] Bioch. J. 7, 96, 1913.
[18] J. Biol. Chem. 15, 311, 1913.

ing food was found to be as satisfactory for growth and maintenance, and also for the recovery of animals which had begun to decline as a result of unsuitable food, as was the natural milk food.

Meantime, in a series of experiments carried on at Wisconsin Experiment Station [19] **McCollum** and his collaborators had been studying nutrition problems, and had been led to attempt to nourish young rats on diets composed of purified proteins, carbohydrates, fats and mineral salts. They confirmed the results of the earlier investigators as to the impossibility of maintaining life on such diets. Animals so fed lived no longer than those which were left to fast. On the assumption that the failure was due to the lack of some dietary essential a systematic attempt was made to discover and identify the missing factor. A paper by **McCollum** and **Davis** [20] anticipated by a few days the publication of **Osborne** and **Mendel** to which reference has been made. These investigators found that while normal growth could be secured with rats on a diet of 18 per cent purified casein, 20 per cent lactose, 5 per cent butter-fat, a salt mixture made up in imitation of the mineral content of milk, and starch to make 100 per cent, no growth could be obtained if the butter-fat was replaced by lard, olive oil, or other vegetable oils. Ether extract of egg yolk was found to have the same stimulating effect as butter fat. They conclude: "The fact that a rat of 40 to 50 grams in weight can grow normally during three months or more on such rations (i. e. those containing neither butter-fat nor egg) then cease to grow but maintain its weight and a well-nourished appearance for weeks, and then resume growth on a ration containing certain naturally occurring foodstuffs would lead one to the belief that on these mixtures of purified food substances the animals run out of some organic complex which is indispensable for further growth but without which maintenance in a fairly good nutritive state is possible."

The conception of a food constituent which is present in natural foods in amounts too small for chemical indentification but which is necessary for the preservation of health had been arrived at from quite a different starting point by another group of workers. The disease of *beriberi*, so common among the poor classes in the East, had been shown by **Eijkman** in 1897 [21] to follow from a restricted diet of "polished" or highly milled rice, whereas when the food consisted of home-milled rice, in which the germ and pericarp is retained,

[19] Wisc. Agric. Exp. Sta. Res. Bull. No. 17, 1911.
[20] J. Biol. Chem. 15, 167, 1913.
[21] Arch. f. path. Anat. 1488, 523, 149, 197.

there is no trouble from the disease. **Eijkman** found that poultry fed on polished rice developed a polyneuritis in which the symptoms were very similar to those observed in human beriberi, and finally died if the diet was continued, but that a prompt cure could be effected by feeding the rice-polishings. He suggested that some substance was removed in the polishings which was necessary to overcome the injurious effect of a harmful factor in the diet. **Fraser** and **Stanton** [22] concluded that the disease was due to a lack of some substance of high physiological importance, the presence of which is essential to the maintenance of health. Scurvy was another disease which was recognized as due to a deficient diet, and a very considerable amount of literature was in existence dealing with the remarkable preventive and curative power of fresh fruits and vegetables as compared with preserved foods.[23]

To the curative substance which he isolated from the rice-polishings **Funk** [24] gave the name of "vitamine" in 1912, and at the same time formulated the hypothesis that the failure to promote normal growth in *young* animals by proteins containing sufficient amino acids to maintain *adult* animals in nitrogen equilibrium, is due to a deficiency in vitamins. A deficiency in vitamins produces also a predisposition to many other diseases, among which is rickets.

Osborne and **Mendel** [25] verified and extended their earlier researches on the growth-promoting properties of butter and demonstrated very conclusively that the essential factor was concentrated in the fat. They confirm **McCollum** and **Davis'** observation as to the efficiency of egg-yolk in promoting growth, and mentioned that all fats are not alike in this respect. While recognizing the importance of **Funk's** work they were hesitant at that time about accepting his hypothesis. "It is still rather early to generalize on the rôle of accessory 'vitamins' when the ideal conditions in respect to the familiar fundamental nutrients and inorganic salts adequate for prolonged maintenance are not completely solved. Speculation is quite justifiable in so far as it directs attention to a new phase that needs to be taken into account." **Funk** has expressed the belief that the substance which promotes growth and must be present in order to avert the cessation of growth, which we have described to occur after a

[22] Studies from Inst. for Med. Res. Fed. Malay States, No. 12, The Etiology of beriberi, 1911.

[23] For Summary, see **Cooper**, Brit. Med. J. No. 2727, p. 722, 1913, and **Funk**, Ergeb. d. Physiol. 13, 125, 1913

[24] Brit. Med. J. 1912, 11, 787, J. State Med. 20, 341, 1912. Ergeb. d. physiol. 13, 125, 1913. Bioch. Bull. 1915, 4, 304.

[25] J. Biol. Chem. 16, 423, 1913.

certain period of successful growth on our earlier dietaries, is either identical with, or analogous to, the vitamin which plays the rôle of an antiscorbutic substance. For this we can as yet find no compelling evidence. Certainly the nitrogen-free butter-fat, so successful in remedying our growth failures, contains no substance chemically related to the nitrogenous products which have lately been credited with this unique physiological efficiency. Furthermore it is well to bear in mind that it is not improbable that the antineuritic and antiscorbutic constituents of foods are not identical with the substances alleged to assist in maintaining body weight."

Considerable uncertainty existed at this time as to whether the growth-promoting substance was to be regarded as identical with antineuritic and antiscorbutic factors. **Stepp**[26] expressed the belief that there is more than a single class of unknown accessory substances necessary for prolonged maintenance of an animal, but he employed only solvents for lipoids in preparing his foodstuff. **Voegtlin** and **Towles**[27] noted that the extracts of autolyzed spinal cord were antineuritic, yet were unable to reëstablish normal metabolism, i.e. restore body weight. **Hopkins**[28] remarks in 1914, "It is uncertain as yet whether the substance necessary for growth is the same as that which can cure the neuritis which develops in fowls fed on polished rice, and the absence of which from a diet is supposed to be responsible for the disease beriberi." **Funk** and **Macallum**[29] found that butter-fat had no beneficial effect on pigeons suffering from polyneuritis, and that young rats and pigeons were unable to live on diets of pure casein, sugar, starch, agar (for roughage), mineral salts, and butter-fat, even when the latter made up as much as 30% of the diet. Addition of from 2–6% of dried brewers' yeast remedied the defect so that good growth and maintenance were obtained. They concluded that "the growth-promoting factor is beyond question contained in the yeast," but were uncertain as to whether there were one or more growth-stimulating components in the yeast, or whether the yeast alone without butter would produce normal growth. **McArthur** and **Luckett**[30] were also unsuccessful in attempting to feed mice on casein, starch, lactose, and mineral salts or protein free milk, all of which had been extracted with alcohol and ether, supplemented with butter to the extent of 25%, but obtained good results when less than 3%

[26] Z. Biol. 62, 405, 1913.
[27] J. Exp. Pharm. 5, 67, 1913.
[28] Ann. Repts. on Prog. of Chem. 1914, 210.
[29] J. Biol. Chem. 1915, 23, 413.
[30] J. Biol. Chem. 1915, 20, 161.

of the alcohol-ether extract of egg-yolk was incorporated in the diet. They proved that the essential substance was neither lecithin, cephalin, cerebrosides, cholesterol, or fat, and suggested that it might be **Funk's** vitamin, but were unable to account for the discrepancy between the results and those of **McCollum** and **Davis** and **Osborne** and **Mendel** with regard to the efficiency of butter-fat.

McCollum and his associates were now engaged in a study of the nutritive properties of the individual grains.[31] In the case of wheat it was found necessary to supplement the grain by addition of protein, inorganic salts, and a growth-promoting fat (butter-fat), but when this was done the diet proved satisfactory for growth, maintenance, and reproduction. They next turned their attention to rice, expecting similar results, but to their surprise they found that polished rice, even when supplemented with protein (casein), butter-fat, and a suitable salt mixture, not only failed to induce growth in young rats, but permitted the development of polyneuritic symptoms. The possibility of a toxic principle in the rice, as suggested by **Caspari** and **Moszkowski,**[32] was eliminated by the fact that no improvement could be detected when the amount of rice in the ration had been reduced to one-half, the deficit being made up by dextrin. Comparison with the earlier experiments in which good health and growth had been secured in animals fed upon purified protein, starch, milk sugar, butter fat, and inorganic salts, suggested lactose as the only missing component constituent in the rice diet, and experiment showed that when 10 per cent of Merck's lactose replaced an equivalent amount of polished rice there was marked improvement. **McCollum** and **Davis** concluded therefore that the lactose carried a second factor essential for normal nutrition during growth, distinct from that present in butter-fat and egg yolk. This new factor, now known as vitamin *B*, is present in milk, and being soluble in water but apparently not in fats, remains in the skimmed milk where the butter-fat is separated. It is absorbed by the lactose and can only be removed from this by very careful recrystallization, so that rations containing commercial lactose would contain an abundance of this substance, as would also the "protein-free milk" of **Osborne** and **Mendel.** It was found to be present also in rice polishings, in water extract of egg yolk, and in

[31] Wisc. Ag. Exp. Sta. Res. Bull. 17, 1911; **McCollum** and **Davis,** Proc. Soc. Biol. Chem. J. Biol. Chem. 14, XI, 1913; **Hart** and **McCollum,** J. Biol. Chem. 19, 373, 1914; **McCollum** and **Davis,** J. Biol. Chem. 20, 415, 21, 615, 1915. See also **McCollum,** Newer Knowledge of Nutrition (1919), pp. 20 & ff. **McCollum** and **Davis,** J. Biol. Chem. 23, 181, 1915.

[32] Berl. klin. Wehnschr. 50, pt. 11, 1515, 1913.

water or alcohol extract of wheat embryo, observations which sufficed to clear up the mystery of the conflicting results referred to above.[33]

Funk's vitamin hypothesis included scurvy as one of the deficiency diseases, an etiology which is almost universally accepted, and the rapidly accumulating evidence concerning the antiscorbutic properties of foodstuffs has proved conclusively that there is a third vitamin concerned, which **Drummond**[34] has designated as "water soluble *C*," following the nomenclature of **McCollum** and **Kennedy.**[35] A more detailed study of the work leading to the recognition of this factor will be given in the chapter on scurvy.

While there is still some difference of opinion as to the part played by vitamins in connection with certain specific diseases, the impossibility of maintaining health on a diet lacking either *A, B,* or *C* is generally regarded as firmly established. As an example of the very small minority who, in the face of overwhelming evidence, persist in attempting to controvert the vitamin hypothesis we may quote **Rohmann.**[36]

Rohmann asserts that accessory foodstuffs are not necessary for the continued maintenance of fully grown animals, and that if long familiar nutrients are suitable in quality and quantity nothing further is essential in the ration.

"The assumption that some unknown substances are indispensable for growth is a convenient device for explaining experiments that result in failure — a device that becomes superfluous as soon as the experiment succeeds."

As evidence against the indispensability of vitamins he cites a new series of experiments on growth and maintenance of white mice fed with artificial mixtures of food supposedly free from these so-called accessory substances. In many of his earlier experiments he used yeast to impart a suitable texture to the food mixtures. Since yeast is now recognized as an efficient source of vitamin, in his latest experiments he has avoided this possible criticism by using starch digested by purified diastase, with baking powder as leavening agent.

[33] **McCollum** and **Davis,** J. Biol. Chem. 23, 231, 1915.
[34] Lancet, 1918, 11, 482.
[35] J. Biol. Chem. 1916, 24, 493.
[36] Kunstliche Enrahrung u. Vitamine, Berlin, 1916.

The diets used by him were as follows:—

Diet I		Diet II	
Egg white	5 gm.	"Kalzose"	2 gm.
Egg white iron	2	Casein iron	20
Potato starch (predigested)	20	Potato starch (predigested)	25
Potato starch (raw)	25	Potato starch (raw)	90
Wheat starch	90	Wheat starch	20
Dextrose	5	Dextrose	24
Margarine	10	Lard	66
Salts	3	Salts	5
Baking Powder	5	Baking Powder	5

The foodstuffs in diet 2 were either treated in alcohol without warming, or were heated three hours at 120–150 C.

The Kalzose used was a commercial preparation of casein combined with calcium, and still containing some milk sugar and doubtless other products present in milk. In this diet 0.2 g. of "Merck's purified diastase," washed with alcohol, was used to digest 20 g. of starch.

Rohmann states that with Diet 1 five young mice having an average initial weight of 6 g. were successfully reared. Addition of casein gave still more successful results, for a third generation was obtained. He states that he has succeeded in rearing young mice and subsequently maintaining them, but these results were frequently induced by added extracts of yeast, by small quantities of milk, or with "Filtrateiweiss," a product "principally composed of the proteins which remain in solution after the precipitation of the casein of milk," and which was supposed to have been rendered vitamin free by heating or by extraction with cold alcohol.

Rohmann's work has been severely criticised by **Osborne** and **Mendel** [37] who point out that it is doubtful whether either of the methods selected by **Rohmann** can be depended upon to exclude all traces of vitamins.

"The thesis that a successful, i. e. positive, experiment in nutrition is far more significant than a negative one is doubless valid. On the other hand, in dealing with substances which, like the alleged vitamins, are potent in surprisingly small amounts, the burden of the proof with respect to the complete absence of effective substances so widely distributed among the natural foodstuffs falls on those who deny the need of them." [38]

[37] J. Biol. Ch. **31**, 149, 1917.

[38] The Vitamine Manual by **Eddy** (Baltimore, 1921) contains a bibliography on vitamins. See also The Vitamines, by **Funk**. Translation from second German edition by **Dubin**.

CHAPTER II

EXPERIMENTAL METHODS

THE preceding chapter explains how it was that so important a factor in nutrition as the vitamins are now believed to be, could have escaped recognition for so long a time, and follows in some detail the gradual development of the modern conception. In order that we may be better satisfied as to the validity of this theory however, a careful examination of the experimental evidence forming its basis is necessary. The present chapter is devoted to a description of the experimental methods employed.

As we have already pointed out, it was a matter of common observation that certain restrictions in the diet were followed in some cases by definite diseases, beriberi and scurvy, and in others by general failure. As knowledge of the familiar constituents of food became more extensive experimental conditions were improved and causes and results could be connected more accurately. In this way the first information about vitamin *A* was arrived at by accident, as it were, in the course of investigations designed to solve other problems, while the direction in which the investigation of the antineuritic vitamin should proceed was indicated by **Eijkman's** observation that the polyneuritis produced in fowls by a diet of polished rice was closely analogous to, if not identical with, the disease of beriberi in man.

Vitamin research has been carried on almost entirely with the use of two or three types of small animals as subjects;[1] — rats and occasionally mice [1a] for the study of the factors *A* and *B*, pigeons and fowls for work on the antineuritic principle (although cats [2] have also been recommended as very satisfactory for the purpose), and guinea pigs and in a few cases monkeys [3] for observations on *C*.

The advantages of working with the smaller animals are obvi-

[1] **Hume** recommends guinea pigs as a substitute for rats in testing for *A* in non-fatty foodstuffs. Biochem. J. 1921, 15, 30.

[1a] **McArthur** and **Luckett**, J. Biol. Chem. 20, 161, 1915. **Rohmann**, F. Ueber kunstliche Ernahrung und Vitamine, Berlin, 1916.

[2] **Voegtlin** and **Lake**, Am. J. Physiol. 47, 558, 1918.

[3] **Chick, Hume** and **Skelton**, Lancet 1918, ii, 735.

ous; the greater ease of caring for them and preparing their food supplies, an arduous task at the best; the simplification of the necessary measurements and analyses; the possibility of working with larger numbers of animals and hence reducing the error from individual variations; and finally the comparatively short time required to enable such animals to reach maturity, making it possible to observe and complete the record of two or three generations within a reasonable time.

The rat is particularly satisfactory for the majority of investigations. Its span of life is about three years; a rat of this age being said to be comparable to a man of ninety [4] although **Slonaker** [5] has kept white rats alive for more than a thousand days. It has a gestation period of only 21 days and the young are weaned at the end of 25 days. The female usually produces her first litter at the age of about 120 days, and will as a rule have five litters by the time she reaches the age of 14 months, which age marks the end of her fertility.[6] Moreover it is omnivorous, and hence adapts itself more easily to a variety of diets than do many species, and, from the many records of animals kept in vigorous health on a suitable diet over long periods of time, it appears to suffer less from confinement than larger animals necessarily must. On the other hand the rat appears to be entirely immune from scurvy, and so for observations on scorbutic and antiscorbutic diets the highly susceptible guinea pig is used instead, while the extreme promptness with which pigeons and fowls succumb to polyneuritis and respond to anti-neuritic treatment renders them peculiarly suitable for experimentation in this direction.

In general, it seems justifiable to assume that a factor which is essential in the diet of one species of animal will be highly advantageous if not equally indispensable for another. The close similarity between the symptoms of polyneuritis in fowls and in cats and dogs and of beriberi in man, of scurvy as observed in the guinea-pig, in puppies, and in human beings, and the fact that the eye-disease so often appearing in animals on a diet deficient in *A* has also been observed repeatedly in children on a diet lacking fat, would appear to confirm this conclusion. Moreover the curative measures proved efficient for the lower animals have produced similar results in human cases. **McCollum** says: [7] "A sufficient

[4] **Donaldson:** Comparison of the white rat with man in respect to the growth of the entire body. Boas Memorial Volume. N. Y. 1906.

[5] **Slonaker,** J. An. Behav. ii, 20, 1912.

[6] **McCollum,** Newer Knowledge of Nutrition, 1919, 14.

[7] **McCollum,** Newer Knowledge of Nutrition, 1919, 15.

number of comparable experiments have now been conducted with several species of animals to make it appear certain that the chemical requirements of one species are the same as that of another among all the higher animals. The requirements with respect to the physical properties of the food vary greatly. The ruminants must have bulky food with the right consistency, whereas the omnivora (man, pig, rat, etc.) cannot, because of the nature of their digestive tracts, consume enough of such foods as leaves and coarse vegetables, to meet their energy requirements."

McCollum, Simmonds and **Pitz**[8] assert that their experience of over a decade with rats and swine has convinced them that the nutritive requirements for these two species at least are essentially the same. As mentioned above, rats fail to develop scurvy on a diet on which a guinea pig succumbs promptly, nevertheless such a diet does not usually promote growth and well-being in rats.

On the other hand certain investigators have uttered a word of warning. **Steenbock, Kent** and **Gross**[9] say that "though the data (obtained by various investigations) cover a considerable range of nutrients from diverse sources much remains to be accumulated before the human dietitian or the animal feeder — unless willingly assuming the rôle of a speculator — can state what limiting factors are imposed on an animal by subsistence on a ration compounded from various sources. In this biological method of analysis, results are obtained which are not always directly applicable to animals of different species. For instance, it has been found that the requirements of birds for the water-soluble vitamin are far greater than those of the rat. Specific unequivocal data can only be obtained by direct experimentation on the animals in question. In this connection it must not be forgotten that the difference in the anatomical features of the digestive tracts of different animals entirely precludes the possibility of obtaining comparable data of the nutritive value of rations for the ruminant, for example, as compared with the rat. . . . As 'guiding information' for the outlining of future work much of value can, however, be obtained by the use of the rat as the experimental animal. When once the dietary properties of various naturally occurring foods have been ascertained individually, it will in many cases be possible to forecast with a considerable degree of certainty the nutritive value of combinations of these. In any case the situation is far from being simple as is especially the case of protein supplementation where the eighteen amino-acids must be considered as the

[8] **McCollum, Simmonds,** and **Pitz,** J. Biol. Chem. 30, 14, 1917.
[9] **Steenbock, Kent** and **Gross,** J. Biol. Chem. 35, 62, 1918.

physiological units, many of which by the inability of the body to synthesize them, can act as growth determinants."

Hart, Halpin and **McCollum**[10] found a striking difference in the mineral requirements at least and possibly in other nutritive needs of chickens as compared with mammals. In this connection they remark that in investigations involving rats, swine, and cattle, there has been constant agreement among these three species in the effects produced by the several rations studied. There may have been some slight differences between the resistances of swine and rats to certain rations, but on the whole substantial agreement has resulted. It does not follow, however, that this would be true with every ration that may arise for investigation nor that the requirements for the normal factors of nutrition be exactly identical among all species of animals. For example, chickens behave entirely differently from either rats or swine on rations limited to corn or wheat grains and their products. Chickens started at half normal weight can maintain themselves and even make slow growth and produce fertile eggs on rations restricted to gluten feed, calcium carbonate, and either corn or wheat meal, while swine and rats on this same food suffer serious loss of weight and on the wheat ration develop symptoms resembling those of polyneuritis. Later work showed[11] that younger birds tolerate wheat much less effectively than do more mature fowls, requiring addition of casein and *A* vitamin, as well as adjustment of the mineral content of the ration, whereas in the case of corn, additions of casein and common salt were sufficient to make it a satisfactory food for baby chicks.

The experimental methods adopted by **Osborne** and **Mendel** in their early work[12] will serve as typical of most of this class of work. The experimental animals were white rats. These were kept in metabolism cages modelled after those described by **Henriques** and **Hansen.**[13] Where it was thought desirable to collect the feces and urine for analysis this was arranged for by resting the cage on a large glass funnel from which the liquid trickled down into a bottle containing chloroform and boric acid, while the solids were retained by a grating in the funnel. In later experiments the funnels were dispensed with, the cages being placed over a frequently changed sheet

[10] **Hart, Halpin and McCollum,** J. Biol. Chem. 29, 57.
[11] **Hart, Halpin and Steenbock,** J. Biol. Chem. 31, 415.
[12] **Osborne and Mendel,** Carnegie Publication 156, 1912.
[13] **Henriques and Hansen,** Zeitschr. f. physiol. Chem. 43, 418, 1904.

of absorbent paper upon an enamelled tray or pan. For the most part, however, the analysis of the excreta is unnecessary in work on vitamins.

The food was mixed into a paste with melted lard to prevent the animals from scattering it as they ate. **Osborne** and **Mendel** say:[14] "The inclusion of 20 to 45 per cent of fat in the diet — a condition necessitated by the requirements of the experiment as outlined — seems like an excessive amount, nevertheless the utilization appears to be satisfactory and attempts to devise less objectionable modes of feeding have been unsuccessful in our hands."

In order to avoid frequent weighings of food, this was introduced into a glass cylinder about 25 cm. in length and 3 cm. in diameter. A rubber stopper was inserted into one end which could be moved forward like a piston head and the food expelled from the other end of the cylinder into the food receptacle. The exit end of the cylinder was kept stopped when the food was not being expelled and the entire apparatus with its food content was kept in an ice-box which made possible its preservation, for long periods without deterioration of the diet. The food eaten can thus be renewed at intervals and the quantity fed determined when desired by ascertaining the loss of weight of each food tube.

Great care was taken to secure optimum conditions of temperature, etc., since rats are sensitive to marked changes. To ascertain whether these conditions were suitable a control series of animals on what was supposed to be an entirely satisfactory diet of ground dog biscuit and lard were kept under observation and their average growth and development compared with that of the group on the experimental diets. **Donaldson's** study of growth in the rat was also used as a standard of normality. From their observations they concluded that neither caging nor monotonous diet reacted unfavorably upon the health and vigor of the animals.

While they were accustomed as a general procedure to add a small quantity of agar-agar to the food mixtures in order to provide roughage and facilitate evacuation, they succeeded in maintaining rats for over a year without this or any other indigestible constituent in the food.

In **McCollum's** work the rats were kept in cages two feet square and twenty inches high, made of wire netting nailed to a wooden frame. The entire floor space was covered by a square pan of galvanized iron which fitted snugly. In the pan clean wood shavings covered the bottom. The food and water supply was suspended so as not to become mixed with the shavings. In this way a good oppor-

[14] **Osborne and Mendel**, l. c. Pt. 2, p. 61.

tunity was provided for ventilation and exercise. Several rats were always kept together.[15]

Mitchell and **Nelson** criticize the use of wood shavings, which they replaced by paper excelsior. They say:[16]

"We cannot agree with the statement of **McCollum** and **Davis** that they do not look upon the consumption of a small amount of wood fibre as objectionable to any greater degree in this type of experiment than is the feeding of agar-agar. Also, the availability and nutritive value of the nitrogenous and mineral substances of the wood cannot be as lightly disregarded, we believe, as they have been by these investigators."

The conditions and technique made use of by **Hopkins** may be taken as representative of the methods of the English investigators.[17]

His experiments were all concerned with young rats at a stage when rapid growth is normal. Their initial weight was mostly from 35 to 50 grams, a stage when weight is normally doubled on an efficient dietary in 20 days or less. The animals, unless otherwise mentioned, were always fed two in a cage, bucks being paired with bucks and does with does. When the amount of food eaten is to be determined, this grouping has the disadvantage that an average for the two animals must be accepted, but when food is given in excess of the quantity required, this is not an important point, and any disadvantages are more than counterbalanced by the fact that quite young rats progress more normally when they have a companion than when kept singly. Great attention was given to the maintenance of a uniform temperature, this being recorded by a registering thermometer. In particular a constant temperature for day and night was carefully secured. The rats were kept in round wire cages somewhat similar to those used by **Henriques** and **Hansen** and by **Mendel** and **Osborne**, though shallower and of wider mesh. They stood in trays filled with sawdust, over which was a layer of filter paper. Rats tend to eat both sawdust and paper, so the cages were raised upon legs four inches high. Two basal diets were used, differing only in the protein they contained. In one the protein was Merck's pure casein prepared by **Hammersten's** method, in the other it was the commercial casein preparation known as "Protene." No roughage was provided, as with rats **Hopkins** considers it unnecessary.

The composition of the dry food mixtures was the following:

	Pure casein mixture	"Protene" mixture
Protein	22%	21.3%
Starch	42.0%	42.0
Cane Sugar	21.0%	21.0%
Lard	12.4%	12.4
Salts	2.6%	3.3

The salts added were obtained by incinerating the normal laboratory food on which the rats had been kept when not under experiment and consisted of equal

[15] **McCollum** and **Davis**, J. Biol. Chem. 20, 644, 1915.

[16] **Mitchell** and **Nelson**, J. Biol. Chem. 23, 460, 1915.

[17] **Hopkins**, J. Physiol. 1912, 44, 427.

parts of the ash of oats and dog biscuits. The commercial casein contained 3 per cent of ash, raising the amount of salts in the mixture from 2.6 per cent to 3.3 per cent. The calculated energy value of the pure casein mixture is just over 5 calories per gram, and that of the protein mixture almost exactly 5 calories per gram. A series of estimations in the bomb calorimeter gave mean value of 4.98 for both mixtures. In general, however, the energy values of the diet were directly determined in the mixtures as they were made up for feeding; the protein and starch not being previously dried.

"The protein, starch, and sugar and salts were mixed dry, and the fat rubbed in by hand, the mixture being worked up until of completely uniform composition. It was given to the animals in earthenware vessels. The day's ration for each pair of rats was weighed out dry, and given the desirable consistency in the following way. Half the ration was mixed with enough water to make it into a thin paste, and the remaining half of the dry material was then added little by little, and well stirred in with a glass rod. The final mixture thus obtained was of such a consistency and character that the rats ate it freely, and almost without any tendency to scatter the food. The amount of food given was always in excess of the consumption. Water was supplied in a special vessel. Before the day's ration was administered to either set of rats, each individual of that set which was to receive milk was put into a separate cage, and the measured quantity of milk given as a separate ration. Only after this was consumed — and its consumption never occupied more than a few minutes — were the rats returned in pairs to the original cages. Both sets were then fed simultaneously with the artificial mixture. The composition of the milk was carefully determined from time to time, and the energy content of the solids was occasionally determined in the calorimeter. This scarcely varied from the value of 4.7 calories per gram."

Hopkins found it possible to determine the amount of food eaten with great accuracy. A very small quantity might fall through the bottom of the cage on to the filter paper beneath, but this could always be recovered quantitatively. Absolute accuracy was to be obtained by removing such spilled food at frequent intervals, so as to avoid any soakage with the urine. The amount spilled was never more than a minute fraction of the food eaten, or of that weighed back; and in practice no difficulty was found in dealing with it. At each day's feeding, the food left over from the previous day was carefully removed from the vessel which contained it, the small quantity of spilled food added to it, and the whole dried at low temperature till of constant weight. This weight was deducted from the dry weight of the food mixture as originally weighed out. During periods in each experiment the feces were collected in order to determine the energy content, so that data as to the absorption of the food might be obtained. The greater part of the feces fell through the bottom of the cage on to the paper beneath, and as this lay upon sawdust, any urine passed was rapidly soaked up, so that very little contamination of the feces occurred. No attempt was made to demarcate the feces of the experimental period, but as they were collected for a week, and under precisely similar conditions in the two sets of rats under comparison, such demarcation seemed unnecessary.

Drummond and Coward[18] have worked out a technique for carry-

[18] **Drummond and Coward**, Bioch. J. 14, 661, 1920. See also **Osborne and Mendel**, J. Biol. Chem. 45, 277, 1920–21.

in out tests for vitamin *A*. The basal ration which they employ is composed of

Purified casein	18 parts
Purified rice starch	52
Refined hydrogenated vegetable oil	15
Yeast extract	5
Orange juice	5
Salt mixture	5

They state that small rats of 50–70 grams (4–5 weeks old) should show very little growth at all and should remain stable for a week or two after the slight initial growth. Any considerable increase in body weight in rats of this age on the diet is to be taken as an indication that the basal ration is insufficiently purified. They regard rats of considerably over 100 grams weight as unsatisfactory for testing for this vitamin and prefer to use only rats of between 80 and 120 grams, of which the growth has been suspended for 10–14 days.

Although the experimental methods above described were developed for use with rats only they will serve to indicate the important points to be observed in the case of animals in general.[19]

Certain general considerations are further emphasized in the literature. It is obvious, as **Osborne** and **Mendel**[20] point out, that the experimental conditions, apart from the food, must be such that control animals on a normal diet can grow and maintain themselves over

[19] For methods of handling other species of experimental animals see: **Wells** and **Ewing**, Georgia Agric. Exp. Sta. Bull. 119, 1916, for experiments with pigs; **Osborne** and **Mendel**, J. Biol. Chem. 26, 293, 1916; 33, 433, 1918. **Drummond**, J. C. Bioch. J. 10, 77, 1916, and **Funk** and **Macallum**, *Ibid.* 27, 3, 1916, for experiments with chickens. **Mitchell**, H. H. J. Biol. Chem. 26, 231, 1916, for experiments with mice; **Cohen** and **Mendel**, J. Biol. Chem. 35, 429, 1911, for experiments with guinea pigs.

Hume (Biochem. J. 15, 30, 1921) advocates the employment of guinea pigs for trial, in testing the vitamin *A* value of non-fatty foods, when for any reason rats are unsuitable for the purpose. Hume fed guinea pigs with a mixture of oats and wheaten bran ad libitum, but weighed. To this was added the food to be tested in weighed or measured quantities; the residues were either fed by hand or were measured and the quantity actually consumed was calculated. Whenever necessary, extra antiscorbutic in the form of orange juice was added. Growth took place when green cabbage (raw or steamed), green cabbage juice, hay and milk (raw, heated or dried) were added to a diet of oats and bran and water. The growth varied according to the size of the ration given. Raw white cabbage, white cabbage juice, swede juice, orange juice, onion or germinated peas produced little or no growth. The growth-promoting accessory substance in these experiments corresponds in its distribution among foodstuffs with the known distribution of vitamin *A*.

[20] **Osborne** and **Mendel**, J. Biol. Chem. 13, 234, 1913.

a satisfactory period of time in a perfectly normal manner. In this connection it should be noted that a "normal" growth curve is not complete proof of satisfactory nutrition. **McCollum, Simmonds and Parsons** [21] observe that when the faults of a diet are of a certain degree of magnitude, animals with a fair amount of vigor may grow at the normal rate and reach full adult size and yet not be in a state of optimum physiological well being. If continued on such diets, they may be tardy in maturing sexually, or may be capable of but a fraction of the fertility of the vigorous, well-nourished individual. It has likewise become apparent that animals which have grown at a satisfactory rate, appear vigorous, and produce a few young at or near the usual age, may fail to maintain fertility to the age normal for the species. In all cases where the diet is below the optimum in character the signs of involution characteristic of old age appear earlier than in the well nourished. These several variations from the normal in the usual functions of the adult animal serve as valuable criteria as to the state of nutrition and are more refined than the simple observation of the growth curve.

McCollum and **Davis** [22] are even more emphatic, urging that in their experience even normal growth to the normal adult size and continued maintenance does not necessarily indicate perfect nutrition. Only when animals reproduce and nourish their young normally, and repeat this at normal intervals, can it be said that the ration is physiologically sufficient. And again, [23] "Growth to the normal adult size at the usual rate and continued well-nourished appearance is not sufficient evidence that a ration is fully adequate. Only when normal reproduction and rearing of the young is repeated at normal intervals can a ration be said to be physiologically sufficient."

It follows therefore that only in those cases where the experiments have extended over a sufficient period of time to cover the life history of at least two generations can the evidence be regarded as entirely trustworthy.

The necessity for long continued experiments before any adequate conclusions can be drawn has been frequently emphasized by various writers and repeatedly demonstrated by experiments in which apparently satisfactory growth over a considerable period of time had ultimately resulted in decline and complete failure.

Hart, McCollum, Steenbock, and Humphrey [24] in their study of

[21] **McCollum, Simmonds** and **Parsons,** J. Biol. Chem. 37, 159, 1919.

[22] **McCollum** and **Davis,** J. Biol. Chem. 20, 416.

[23] *Ibid.* 21, 620.

[24] **Hart, McCollum, Steenbock** and **Humphrey,** Un. Wis. Agric. Exp. Sta. Res. Bull. No. 17, 1911.

the physiological effects on growth and reproduction of cows on rations derived from a single plant carried on experiments over a period of three years, and conclude that unquestionably the physiological value of a ration is largely dependent upon its chemical constituents, but the usual determinations made on feeding materials do not reveal the character or manner of combination of many of the constituents. Consequently, according to these investigators, the physiological value can be determined, in the present state of our knowledge, only by long continued observations of the reaction of the feed on the animal."

Osborne and **Mendel** confirm this.[25]

"The necessity of long continued experiments calls for particular emphasis. Physiological alterations dependent upon the gradual depletion of a small store of essential tissue material may manifest themselves with extreme slowness; and the fact that a satisfactory nutritive balance can be maintained for a week or two or even a month in some cases is no guarantee of either the ultimate success of the dietary or of the impossibility of a decline owing to the inappropriate exhibition of an essential ingredient — a deficiency in some essential ingredient may not make itself manifest for a long time, and even then be difficult to detect; for many other causes than defects in the food may lead to the decline or death of animals during experiments lasting over many months. It is only when the failure to be maintained is the invariable outcome of the prolonged feeding on a given diet that we are justified in assuming that the diet is in some way inadequate, and then only when a prompt and complete recovery ensues when the diet is changed to one that is known to be in all respects sufficient.

. . . If we had been content to discontinue the experiments after a reasonable period many of the declines evidently associated with imperfections in the dietary and readily checked by a change in feeding would have escaped attention."

A difficulty which is met with at the outset of all experimental work is the great variability in animals of the same species with regard to growth, impulse and vitality. This has been repeatedly emphasized by the various experimenters. **Osborne** and **Mendel**[26] noted that when rats were kept on a diet of purified protein, lard, starch, and protein-free milk, growth ceased after sixty days in some cases while others continued to grow for a hundred days or more. They report the case of one rat among hundreds tested on inadequate lard diets, continuing to thrive for the exceptional period of 252 days before the inevitable failure ensued.[27]

[25] **Osborne** and **Mendel,** J. Biol. Chem. 13, 233, 1912–13.

[26] **Osborne** and **Mendel,** J. Biol. Chem. 15, 313, 1913.

[27] **Osborne** and **Mendel,** J. Biol. Chem. 20, 383, 1915.

McCollum and **Davis**,[28] state that rats may grow well for three or four months on fat free diets of purified casein, dextrin, milk sugar, agar-agar, and salts from reagents, the period of growth depending on the stamina of the individual. They note that many make no growth at all; others, and indeed the greater number will make about half the normal growth, while a few individuals of exceptional vitality will grow at the normal rate. By careful attention to the selection of breeding stock during several generations they were able to greatly increase the percentage of individuals which approximated normal growth under these conditions. Further they state that the domestic rat has been kept under conditions where inferior specimens have survived and propagated until the general vitality of the strain is low. This fact, however, does not detract from the value of observations with rats of improved vitality. What we are seeking to learn is the chemical powers of the cells of the mammalian organism, and in order to do this we must eliminate just as far as possible all other factors which can influence the animals' tendency to grow. The power of growth is not the same in all individuals of the same species, or even of the same family, yet we must admit that the stronger ones are the more normal, in that all their physiological processes are functioning with greatest smoothness and power. In nutrition studies it is of the greatest importance that success or failure shall turn upon a single factor. Obviously where animals with the most exceptional vigor are not used, even though a considerable number be employed, the conclusions from the experiments may be entirely misleading. If success in growth or reproduction is to turn on the single factor of the lack of a certain complex or complexes in the ration, **McCollum** and **Davis** assert that we must know the animals are so vigorous that they are accomplishing everything which any individual of the species is prepared to accomplish, as far as the chemical character of the ration will admit.

McCollum, Simmonds and **Parsons** [29] call attention to the same point, observing that when an animal in infancy is fed a diet unsatisfactory in any respect it does the best it can under the circumstances. There is a variable factor in the vitality with which the individual is born, which in some measure will determine its ability to utilize food faulty in any respect. Only in this way can an explanation be provided for the great variation in the ability of several individuals in the same experimental group to grow on the same diet. Since it is not possible to determine the extent to which any individual possesses natural vigor, an experiment should be conducted with several animals so that one or more will be of great vitality. The elimination of the unfit in the breeding stock is important for this reason.

Funk and **Macallum** [30] similarly note:

Experience demonstrates that there are wide variations depending on the constitution of the individual rats. Every rat taken individually is not suitable for this class of work. As a matter of fact in one case, 80 per cent of the

[28] **McCollum** and **Davis**, J. Biol. Chem. 20, 641, 1915.

[29] **McCollum, Simmonds** and **Parsons**, J. Biol. Chem. 37, 159, 1919.

[30] **Funk** and **Macallum**, J. Biol. Chem. 27, 51, 1916.

rats purchased from dealers were rejected on account of physical defects not apparent before the initiation of the experiments. A second complication is a diminished resistance to infection which follows the use of all artificial diets. The meager knowledge we possess of the pathological conditions in rats may lead to a condemnation of the diet; whereas actually the condition could be remedied without change of diet, if we were able to recognize its nature. As an example of this, rats on artificial diets frequently contract an eye infection which can be treated with a certain degree of success by an application of a few drops of zinc sulphate solution. If untreated this condition is accompanied by loss in weight, becomes acute, and terminates fatally.

Funk recommends the use of rats specially bred for the purpose in the laboratory. He [31] laments that the animals furnished by dealers seemed to lack that power of resistance which would make them suitable for feeding experiments. This was manifested by a loss of hair on being placed in the metabolism cages, and early death before the experiments were well under way. This difficulty was promptly eliminated when rats bred in Funk's laboratory under proper dietary conditions were substituted. The animals which were used for breeding purposes thrive best when kept on a diet of oats, bread, condensed milk, and yeast. The success of this diet was evidenced by the production of better animals from the physical as well as from the experimental standpoint.

In order to determine the degree of variability among young rats on a normal diet, **Hopkins** [32] transferred fifty rats from the regular diet of the laboratory — dog biscuit and oats — to a diet of bread and milk, and noted the time required for them to double their weight. The change of diet was made in order to reproduce more exactly the conditions under which the usual laboratory experiment is carried on, a sudden change of diet at the beginning of the experiment being inevitable in these cases.[33]

Half the animals used were bucks, and half does. The most striking result of the test was its demonstration of a much greater regularity in the variation of the bucks. The initial weights of the animals fed, while mostly near to the average of 50 grams, showed in certain cases a considerable departure from this (46 to 63 grams). Nevertheless, the periods of weight doubling in the case of bucks departed comparatively little from the mean value. . . . In the case of 19 rats out of 25, or 76 per cent, the completion of the weight-doubling period occurred within three consecutive days (thirteenth to the fifteenth). Two other rats may be grouped with these, one completing its period on the 11th, and one on the 16th day. Of the remainder two doubled their weight on the 20th, one on the 21st, and one on the 26th day. Over 80 per cent therefore showed periods near to and normally grouped round the mean.

[31] **Funk,** J. Biol. Chem. 27, 1, 1916.

[32] **Hopkins,** J. Physiol. 44, 430.

[33] **MacArthur** and **Luckett,** J. Biol. Chem. 20, 164, point out that whenever any food mixture was given to a mouse accustomed to an ordinary diet of corn, dog biscuit, sunflower seeds, carrots, and meat, there was a short period of slower growth, or even no growth, followed by nearly normal growth if the food mixture was complete.

On the other hand the does showed much greater irregularity. Eight animals, or 32 per cent, grew at about the same rate as the bucks; completing their weight doubling between the 13th and the 16th days; 6, or 24 per cent, completed it round about the 20th day; another five required about 30 days; and the remaining four grew still more slowly; one animal, though showing no signs of ill health, had not doubled its weight by the 56th day.

A longer comparison further brought out the relative slowness in the growth of the females. Thus on the 52nd day of the experiment the mean weight of the bucks was their mean initial weight multiplied by 3.58. In the case of the does the corresponding factor was 2.63.

Even where the most careful procedure is followed it is necessary to guard carefully against erroneous conclusions drawn from misleading evidence. Speaking from wide experience **Davis** [34] considers many confusing factors to exist in spite of the progress that has been made. Clear-cut results are the exception rather than the rule. An animal may be abnormal and recover without change in the ration, or it may be subnormal and not recover when taken off the experiment. Frequently the individual variations within a group are greater than the variations between groups. Some of these individual abnormalities are due to simple causes. It sometimes occurs that a rat will continually lose weight on a diet supposedly liberal in all respects. Such animals are not infrequently found to be suffering from pulmonary infections, hair balls in the stomach, tumor, etc., the discovery of which explains the unexpected results." [35] If however the complicating factor should happen to remain unknown very inaccurate conclusions as to the diet might be drawn.

Much depends on the condition of the animal at the beginning of the test. It is fairly generally accepted that the animal, especially at maturity, has some power of storing up a sufficient amount of the essential factors to tide over a certain period of deprivation, the length of this period depending on the natural vitality of the animal. **Osborne** and **Mendel** call attention to the possible importance of this factor from the records of the long periods of starvation — over 117 days in the case of the dog — which have been survived successfully.

In another connection, **Wheeler** [36] reports that animals have a surprising power of getting along for a time on a qualitatively inadequate diet. Mice kept upon a ration in which two-thirds of the protein was gelatin and the other third casein, did not show a decline

[34] **Davis,** J. Home Econ. 12, 207, 1920.

[35] **Osborne** and **Mendel,** J. Biol. Chem. 22, 247, 1916.

[36] **Wheeler,** Exper. Zool. 15, 210, 1913.

in body weight for 21 days, although the ultimate decline of every animal fed upon this food showed it to be insufficient; another group made material gains in weight for 17 days on a diet upon which all ultimately lost weight rapidly and died unless the food was changed. Similar observations have been made by other investigators.

Moreover, the minimum requirement of vitamins may vary from time to time. **Drummond**[37] believes that the requirements of the growing rat for *A* become less as the animal approaches maturity and that the amount of this factor necessary to induce normal growth in a rat which has ceased growing on a vitamin-poor diet is inversely proportional to the weight of the animal.

Besides the individual variability already referred to, **Osborne** and **Mendel**[38] report a peculiar seasonal variation which affects their rats. During the late summer and fall over a period of three years, young rats on protein-free milk diets almost invariably fail to grow, while at other seasons on the same diet they make normal growth for about three months. "The failure to make early growth is accompanied by diarrhoea, diminished appetite, and, after three or four weeks, by inflamed eyes which soon develop into a purulent state."

Dietary factors other than the particular one under observation may be ill adjusted. The necessity for recognition and rigorous adjustment of all factors in the diet is strongly emphasized by **McCollum**[39] and by **Osborne** and **Mendel.**[40] Only when we are assured that every factor but one is at its optimum can we successfully trace nutritional success or failure to its source, still less obtain a quantitative measure of the influence of the factor in question. The importance of the protein and mineral constituents apart from the vitamin content is referred to in a later chapter,[41] but these must also be considered in relation to vitamins.

According to **Funk**[42] less vitamin is required on a high protein diet and more when the carbohydrate content of the ration is large.

[37] **Drummond,** Bioch. J. 13, 95; 14, 661, 1920.

[38] **Osborne** and **Mendel,** J. Biol. Chem. 17, 401, 1914.

[39] **McCollum, Simmonds** and **Pitz,** J. Biol. Chem. 25, 111; **McCollum** and **Simmonds,** *Ibid.* 32, 181; **McCollum, Simmonds** and **Parsons,** *Ibid.* 37, 159.

[40] **Osborne** and **Mendel,** *Ibid.* 37, 159. See also **Steenbock** and **Gross,** *Ibid,* 40, 507, 1919.

[41] See Chap. III.

[42] **Funk,** Z. Physiol. 1914, 89, 378; **Funk, Lyle** and **McCaskey,** J. Biol. Chem. 27, 173, 1916; **Funk** and **Dubin,** Sci. 52, 447, 1920; **Braddon** and **Cooper,** Brit. Med. J. 1914, 1909; J. Hyg. 14, 351, 1914; **Theiler, Green** and **Viljoen,** 3rd and 4th Rep. of Director of Vit. Res. Union of So. Africa, 1915, 9–68.

If this is so the percentage composition of the basal ration might have a pronounced effect upon the vitamin minimum.

Complication may be introduced through the presence of a toxic substance in the food, in which case, according to **Hart, Miller** and **McCollum**[43] an additional supply of the essential factors will be required to counteract the injurious effect. Little is known as to the toxicity of common foodstuffs when fed as part of a restricted ration. **Davis,**[44] believes that many of them may be toxic to certain animals if fed in sufficient amounts for a sufficient time. The toxicity of cottonseed is well attested and toxic effects of the whole wheat grain have been reported several times[45] although **Osborne** and **Mendel** dispute this.[46] A substance like magnesium which is not only harmless but essential in proper concentration may be distinctly toxic in larger amounts. That slight toxicity or other injurious character in a ration may easily exist without recognition and thus lead to inaccurate conclusions is brought out by **McCollum, Simmonds** and **Pitz**[47] in another connection.

"A single factor (protein) in a ration may appear to admit of the maximum performance of the animal with respect to growth, without itself representing the optimum amount or character. When this circumstance prevails, it may entirely escape notice, yet if in another ration exactly like it, except that a second factor tends to injure the animal, nutritive failure may result. In such a case as the latter, improvement of the protein factor by the addition of more protein or by the substitution of a better protein, the plane of protein intake remaining unchanged, the animal may make the maximum performance notwithstanding the unfavorable character of the injurious factor of the ration."

Osborne and **Mendel**[48] point out that the problem of dosage for vitamins may be quite different in the case of rats which are supplied with a minimum of the essentials throughout the period of growth and those which have declined through complete deprivation of some requisite food factor.[49]

[43] **Hart, Miller** and **McCollum,** J. Biol. Chem. 25, 241, 1916.

[44] **Davis,** J. Home Ec. 12, 207, 1920.

[45] **Withers** and **Ray,** J. Biol. Chem. 14, 53, 1913; **Withers** and **Brewster,** *Ibid.* 15, 161, 1913; **Crawford,** J. Pharm. Exp. Therap. 1910, i. 519; **Richardson** and **Green,** J. Biol. Chem. 30, 249; **Hart** and **McCollum,** J. Biol. Chem. 19, 373, 1914; **McCollum, Simmonds** and **Pitz,** *Ibid.* 25, 105, 1916; **Hart, Miller,** and **McCollum,** *Ibid.* 25, 239, 1916; **Hart, McCollum, Steenbock** and **Humphrey,** Proc. Nat. Acad. Sc. 3, 374, 1917; J. Agric. Res. 10, 175, 1917; **Hart. Steenbock,** and **Humphrey,** Univ. Wis. Agric. Exp. Sta. Bull, 287, 1918.

[46] **Osborne** and **Mendel,** J. Biol. 37, 595, 1919.

[47] **McCollum, Simmonds,** and **Pitz,** J. Biol. Chem. 25, 111, 1916.

[48] **Osborne** and **Mendel,** J. Biol. Chem. 41, 452, 553, 1920.

[49] **Osborne and Mendel,** J. Biol. Chem. 41, 452, 1920.

There are two feeding methods of testing products for the presence of water-soluble vitamin. One consists in feeding the material to be investigated to animals that have declined on a diet deficient in this food factor. Such tests, however, will not demonstrate the comparative vitamin content of different foods. They are essentially restorative in character. If the outcome is positive in the sense of a renewal of nutritive well-being, the test for the vitamin may be qualitatively successful. However, animals which have suffered a vitamin deficiency may be so badly malnourished or underfed that relatively excessive amounts of the vitamin-bearing food may be required to restore them to normal condition. In that event a failure to promote nutrition by the extract to be tested may be ascribed to the condition of the animal rather than the product. Comparisons of vitamin-bearing foods may not be reliable when they involve attempts to restore nutrition in animals that have suffered to a variable degree from unlike deficiencies.

The other method consists in feeding the supposed source of vitamin to animals which are normal in health and development, and observing whether the product offered supplies what is needed to promote normal growth when the diet affords an adequate supply of all essentials except that to be investigated.

The first of these methods is the one which has been chiefly resorted to in testing for vitamin *A* in plant and animal products, and also very commonly in the study of antineuritic *B*. In much of the more recent work on both *B* and *C*, however, both methods have been used and the results given for comparison.

Sherman, LaMer and Campbell [50] suggest the following procedure for the quantitative determination of the antiscorbutic vitamin. Guinea pigs are fed a basal diet consisting of 59 per cent oats, 30 per cent skim milk powder heated two hours at 110° C., 10 per cent butter-fat, 1 per cent NaCl. The minimum protective dose of antiscorbutic is determined, and in addition the degree of scurvy produced, as measured by the autopsy findings, retardation in growth, and symptoms in life, of a series of animals receiving graduated subprotective doses of antiscorbutic food. When the dosage is calculated per unit of body weight it is possible to distinguish the degrees of scurvy produced for addenda of antiscorbutics differing by 15 per cent or less. The percentage destruction due to a deleterious process is obtained by comparison of the degree of scurvy produced in a series of standard animals fed a similarly graduated series of doses of the treated product. The probable error of the mean in a series of five or more animals is less than 4 per cent.

[50] **Sherman, LaMer and Campbell, Science, Aug. 26, 1921, 176.**

The accurate evaluation of much of the earlier research is difficult on account of the lack of data as to the actual food intake. With reference to this, **Osborne** and **Mendel** [51] say:

> If no account of the food eaten is taken the outcome of the experiments may be wholly misleading. Some preliminary experiments appeared at first sight to indicate a marked superiority of barley proteins compared with those of wheat or rice; but when gains of weight per gram of protein eaten were calculated this superiority entirely disappeared. It is thus plain that experiments which have hitherto purported to show the relative nutritive value of different food products in respect to any one of their constituents have no comparative value unless the amount eaten is known.

Chick and **Hume** [52] criticize most of the research on vitamins on the ground that the consideration of quantity has been overlooked to a large extent in the experimental methods adopted, and that the conclusions drawn have been correspondingly untrustworthy.

In comparing the value of a series of foodstuffs as regards their value in content of some accessory food factors, the first step necessary, according to **Chick** and **Hume**, is to determine in each case the minimum daily dose which will maintain health in the experimental animal and to institute comparison between these amounts. These daily doses should be as large as can be tolerated before it is safe to deny to a foodstuff any value in respect of the particular accessory factor under consideration. Neglect of this simple and fundamental, but admittedly tedious procedure is evident in much published work upon the influence of various treatment upon the antineuritic and antiscorbutic and other properties of certain foodstuffs, and the results obtained are in consequence vague, and may even be erroneous. For example, if a foodstuff is rich in any particular accessory factor and is fed to an animal in large excess of the amount actually required, any destruction of the factor that may take place during heating cannot be assessed and may even remain undetected if the heated material is fed in similar large amount. It is held necessary to determine the minimum required in both cases, and to institute a comparison between these two results before any satisfactory conclusion on this point can be drawn.

The methods used by **Osborne** and **Mendel** and by **Hopkins** for determining the food intake have already received mention in this volume. Where possible, it seems distinctly advantageous to feed the material to the tested separately from the basal ration, so that this

[51] **Osborne and Mendel,** J. Biol. Chem. 34, 523, 1918. See also **Steenbock, Boutwell and Kent,** *Ibid.* 35, 518, 1918.

[52] **Chick and Hume,** J. Biol. Chem. 39, 203, 1919.

can be estimated separately and no error may be introduced should the animal fail to consume the whole ration.

It is evident from the foregoing that the problems of vitamin research are far from simple, and that the correct interpretation of the results requires an extensive knowledge of all the detail of the experiment. It is extremely difficult, as **Davis**[53] has pointed out, to secure uniform results in one laboratory, much more so to evaluate and correlate the results from a hundred different laboratories in all parts of the world.

In an attempt to develop a simple standard test for at least one vitamin, *B*, **R. J. Williams** and **Bachmann** have both made use of the fact that certain yeasts fail to grow satisfactorily in vitamin-free media, whereas on the addition of vitamin, growth takes place at a rate which is at least roughly proportioned to the amount of the vitamin present.

Bachmann[54] noted that Saccharomycetes grow better and ferment more readily in a medium containing small amounts of organic material but vary in their needs for traces of organic matter other than sugar. Some will grow in a solution of inorganic salts and sugar even when introduced in small amounts, as observed by **Pasteur.** A certain yeast isolated from fermenting canned pears was found not to grow in or ferment **Nageli's** solution unless introduced in very large amounts or some organic substance was added. The substances which cause fermentation are those which have been found to be rich in vitamins (especially water-soluble *B*), such as yeast extract, vegetable juices, milk, and cabbage extract, apparently containing the most efficient vitamins for yeast.

It is suggested that in a heavy inoculation some of the cells are dead, or that because the medium is unfavorable some may die, and that from these dead cells soluble substances that are stimulating to growth and fermentation of other cells diffuse out into the medium.

The liquid medium used for testing vitamin requirements of yeast was **Nageli's** solution, composed of 100 cc. distilled water, 10 g. dextrose, 1 g. ammonium nitrate, 0.05 g. calcium phosphate, 0.5 g. potassium acid phosphate, and 0.25 g. magnesium sulphate.

None of the salts were especially purified by recrystallization. Durham tubes were usually used to show gas production and the amount of gas produced, approximately 8 cc. of the medium being placed in these tubes.

[53] **Davis, J. Home Ec. 12, 206.**

[54] **Bachman, J. Biol. Chem. 39, 235–57, 1919.**

Of two strains of yeast examined, one, an aerobic type, was able to cause fermentation in a medium containing little or no vitamins, while the other, which does not produce a surface film and is therefore anaerobic, will not ferment except in the presence of vitamins.

Williams used an entirely different technique, but came to the same general conclusions as to the importance of vitamins in the life-processes of the yeast cell. His method consisted in making a hanging drop culture of a single cell, and noting the rate of multiplication. A single yeast cell can be caused to produce from one to several thousand cells in 24 hours by varying the vitamin content, and it was found that the number of cells produced under given conditions in 18 hours is directly proportional to the amount of vitamin added. Inasmuch as several investigators have used this method or a modification of it, the experimental details are given, although Williams himself has now superseded it by a gravimetric method which he asserts to be more accurate and more easily applied.

The medium used was made up of purified reagents as follows: saccharose, 20 gm.; ammonium sulphate, 3 gm.; potassium dihydrogen phosphate, 2 gm.; asparagine, 3 gm.; calcium chloride, 0.25 gm.; magnesium sulphate 0.25 gm., all made up to 1 liter with distilled water. The media was sterilized at 10 pounds pressure for 10 minutes and kept in the refrigerator.

A suspension of yeast cells was made in about 30 cc. of sterile distilled water. Duplicate test solutions were prepared quantitatively by taking 25 cc. of the synthetic media and 1 to 5 cc. (depending upon the concentrations) of a definite solution of the unknown extract. This was diluted to 30 cc. (using sterile water if needed). The mixture was sterilized and 1 cc. of the yeast suspension added. After mixing, thirty-six drops were made with a fine pen point on a cover slip that had been coated with a very thin film of purified vaseline. This slip was inverted and sealed tightly on a hanging drop slide. Each drop was examined for single yeast cells, the observations were recorded, and the time was noted. The slide was then placed in the incubator at 30° C. and the readings were made again in 18 hours. In case the number of cells in each drop at the end of this period exceeded 75, the determination was repeated using less of the unknown. Similar tests were run on the synthetic media as a control. After correcting for this blank determination, the rate of growth in terms of yeast cells was calculated per gram of the original unknown material. This method was used satisfactorily by **Emmett** and **Stockholm**[55] but **Whipple** found it unsatisfactory.[56]

[55] **Emmett and Stockholm**, J. Biol. Chem. 43, 287, 1920.

[56] **Whipple**, J. Biol. Chem. 44, 175, 1920.

As Williams himself points out the method is far from ideal for general use since some practice is necessary before it can be applied with any confidence, and a personal factor is introduced to quite an extent. Williams' gravimetric method, which he considers preferable, is carried out as follows: [57]

The medium contains

20.0 gm. of cane sugar.	1.5 gm. of asparagine
3.0 " " ammonium sulphate	0.25 " " calcium chlorid
2.0 " " potassium dihydrogen phosphate.	0.25 " " magnesium sulphate

All dissolved in 1 liter of distilled water.

One hundred cc. of this solution are put into a 500 cc. Erlenmeyer flask and a known amount of the solution to be tested is added. The volume is made up to 110 cc., the flask is plugged with cotton, sterilized or pasteurized to kill all vegetable organisms, cooled, and brought to a temperature of 30° C. in an incubator.

A yeast suspension is made by weighing out 0.300 gm. of fresh Fleischmann's yeast (small cake in tin-foil) taken from the center of the cake; this is made into a paste with a very small amount of water and suspended finally in 1 liter of sterile water. 1 cc. of this suspension well shaken and freshly made is introduced into the culture medium with a sterile pipette. By this means 0.3 mg. of yeast is used for seeding.

The flasks are then put into the incubator and the yeast is allowed to grow undisturbed 18 hours at 30° C., when the growth is stopped by the addition of a little formaldehyde solution. If the seed yeast has not been kept in a refrigerator continuously since manufacture, a very rapidly growing wild yeast may appear to a slight extent, floating on the surface of the solution. This should be removed by lifting it from the surface with a small piece of fine copper gauze attached at right angles on the end of a glass rod or tube. This removes practically none of the other yeast, most of which settles to the bottom.

The yeast is then filtered off on a weighed "Gooch" crucible, prepared with paper underneath the asbestos, washed thoroughly with water, finally with a little alcohol, dried for 2 hours at 103° C., and weighed after an hour's cooling. The "Gooch" crucibles cannot be used repeatedly without treatment as the dried yeast loses weight on washing with water.

[57] Williams, J. Biol. Chem. 42, 259, 1920. See also Swoboda, *Ibid.* 44, 531, 1920.

If no vitamin is added to the solution, the yeast produced will be about 2.5 mg. The amount of growth above that of the blank is, within limits, directly proportional to the amount of vitamin put into the solution. The results may be expressed numerically and the number represents the relative purity of the preparation tested or the value of the material tested as a source of this vitamin. The "vitamin number" of a material may be defined as the number of milligrams of yeast produced by the addition of its extract, minus that produced in a control solution, under given conditions and within certain limits, computed to 1 gm. of the original material tested.

Of this procedure **Williams** observes that the vitamin to be tested must be in solution. No method of extraction is outlined. According to **Williams** nothing except vitamin (especially in small amounts) is able to produce more rapid growth, hence the vitamin does not need to be prepared especially free from other materials, as impurities in all probability will have no accelerating effect. The total amount of material added ordinarily will be much less than 0.1 gm. In using this method for vitamin determinations, precautions must be taken that the vitamin is extracted quantitatively from the material in question. Investigators have found that this is difficult to do, especially from some materials. In the handling of the extracts care must be taken that toxic substances are not introduced or formed which will counteract the effect of the vitamin which may be present. This is true also of animal feeding experiments where poisonous substances must be avoided. Treatment of material with acid especially at high temperature or overheating in the dry condition frequently produces something toxic in yeast, while treatment with alkali may destroy the vitamin. In some extracts the vitamin is destroyed to an appreciable extent if kept at the boiling temperature an hour or more. An autolysate of yeast is not a desirable material as a yeast nutrient as inhibiting substances appear to be present. If the amount of growth produced is not proportional to the material added, but falls off with the larger quantity, it is good evidence that something toxic is present, which becomes more potent in the higher concentration and counteracts the effect of the increase in vitamin. This can be confirmed by microscopic examination of the yeast produced, which reveals small granular irregular cells or other abnormalities. It is mainly due to the possible presence of toxic substances that two dilutions should be used for a determination. If toxic substances are present the results from the lower concentrations are more reliable.

A comparison of the vitamin content of various foodstuffs as determined by **Williams** with yeast and by **Osborne** and **Mendel** with

rats gave for the most part fairly concordant results.[58] Where discrepancies existed there are some indications that vitamin *C* might be acting as a second factor in stimulating yeast growth. The yeast test indicates a higher vitamin content in bakers' yeast than in brewers' yeast, whereas the animal tests indicate the reverse. Yeast extracts appear, however, to exert a specific influence on different varieties of yeast, since extract of brewers' yeast was found to be a better stimulant for the growth of brewers' yeast than bakers' yeast extract, while the latter had more influence on the growth of bakers' yeast.

Eddy and **Stevenson** [59] experimented with both the **Bachmann** and the first **Williams** methods of quantitative study of vitamin content, and finally devised a modification of both which they report as satisfactory.

A pair of capillary pipettes is made and calibrated to hold one unit, the unit being arbitrarily chosen, in this case a drop of mercury weighing 0.0108 g. After sterilization these tubes are ready for use. A pure culture of Fleischmann yeast is maintained on an agar slant, a transplant being made to fresh agar 48 hours before beginning the test. At the end of 48 hours as small a portion as can be taken up on the tip of a needle is transferred to 10 cc. of sterile **Nageli** solution (100 c. distilled water, 10 g. dextrose, 1 g. ammonium nitrate, 0.05 g. potassium acid phosphate, 0.25 g. magnesium sulphate) and the tube then shaken for two or three hours in a mechanical shaker. This process does not give absolutely uniform suspensions and occasional clumps are found, but these can be removed for the most part by slow centrifuging and the method has been found satisfactory for results if controlled by a sufficient number of tests. The uniformity of the suspension is tested before use by drawing up with the pipette 5–10 units of the suspension and blowing them out on a glass slide where they are fixed, stained, and the cells counted.

When a fairly uniform suspension is obtained one unit of yeast solution is drawn up into the pipette and one unit of sterile vitamin solution. These are mixed by manipulating the bulb, the pipette is sealed at both ends, and the sealed tube incubated for a suitable interval of time, usually 20 hours at 35° C. At the end of the time the tips are broken at each end of the pipette, a bulb is placed on the large end, and the contents are blown out on a slide, fixed and stained. For a counting slide, **Eddy** and **Stevenson** use an ordinary micro-

[58] **Williams,** J. Biol. Chem. 46, 113, 1921.

[59] **Eddy and Stevenson,** J. Biol. Chem. 43, 295, 1920.

scope slide on which 5mm. squares are etched. The size holds the contents of a pipette, permits ease of operation when counting with the mechanical stage, and allows the content of ten to twelve pipettes to be placed on one slide. For control, another series of pipettes is prepared and filled by drawing up a unit of the yeast suspension without the unit of vitamin. These are incubated and counted in the same manner.

The method was tested with small quantities of **Funk's** antineuritic vitamin as prepared by him, and the results seemed to strengthen the conviction that this is the responsive causative agent in stimulating the growth of the yeast cells.

Experiments with orange juice showed that the cause of the stimulus is nearly quantitatively removed from orange juice and from navy bean extract by shaking these extracts with **Lloyd's** reagent. Since **Seidell** has shown that this reagent removes the curative factor for polyneuritis these results would seem to add to the other evidence that the test is specific for the *B* vitamine.

Tests with guinea pigs showed that the treatment with **Lloyd's** regeant did not remove *C,* in confirmation of the results of **Harden** and **Zilva,**[60] therefore it is assumed that the *C* is not responsible in any way for the yeast stimulation.

In estimating the vitamin content of foodstuffs by this method, the probable range of variation in the suspension used was first determined then five to ten tests were made on the substance under examination, and from these all zero readings were eliminated and the rest averaged. In doubtful cases this was repeated as often as necessary.

Funk and **Dubin**[61] have devised a simplified yeast test which they believe is valuable for determining the approximate vitamin activity of a given food substance, although the presence of inhibiting substances may interfere with the reaction to such an extent that the method cannot be used for comparison of the vitamin content of different substances. They prepare yeast suspension by shaking a loopful of a forty-eight hours' pure culture of yeast in 100 cc. of **Nageli** solution on a shaking machine for three hours. Measured quantities of this suspension are then incubated for twenty hours at 30° in the presence of the unknown vitamin solution, control tubes with a vitamin solution of measured strength being employed. After incubation, the fermentation is stopped by heating the contents of the tubes at 75° for a few minutes, and the amount of yeast is estimated by centrifugalizing in a specially calibrated tube. Using autolyzed

[60] **Harden and Zilva, Bioch. J. xii, 93, 1918.**

[61] **Funk and Dubin, J. Biol. Chem. 44, 487, 1920.**

yeast as a standard they plotted a curve by means of which the vitamin content of an unknown substance can be expressed in terms of the standard. The method is said to be sensitive to 0.0001 cc. of autolyzed yeast, but the test is best arranged so that the amounts of unknown substance used correspond to 0.05 cc. of autolyzed yeast.

Sousa and **McCollum**[62] also introduced certain modifications of **Williams'** early procedure, but their study led them to conclude that the method was of little value to a test for the presence of the antineuritic factor. Instead of a pen for distributing the drops, they used a *statuuner* syringe needle having an opening at right angles to its axis, the needle being attached to a piece of glass tubing by a short piece of rubber tubing. It was found that a fairly uniform distribution of cells could be obtained in this manner.

In order to avoid the difficulty caused by lack of uniformity in development of yeast cells a **Levy** blood-counting chamber was used instead of the cover slips used by **Wllliams.** Comparison of the two methods showed that a more even and reliable result can be obtained by the use of this procedure. They tested the growth-stimulating effect of aqueous extract of wheat germ made by boiling the germ with water. The results showed clearly that there is a stimulating effect of the extracts on the rate of multiplication of yeast and that in general the greater the amount of extract added the more pronounced the acceleration. Similar results were obtained with the use of alcoholic extract of wheat germ.

A set of experiments was made with wheat germ extract treated with 2–4 per cent of sodium bicarbonate and autoclaved, in order to destroy as much as possible of the antineuritic substance. Addition of these extracts exerted a profound stimulating effect on the growth of yeast, but the extent of acceleration of growth was not proportional to the amount of extract added. This germ was used in a feeding experiment with rats to show whether it still contained any appreciable amount of *B*. The ration consisted of casein (18%), treated wheat germ (15%), butter-fat (5%), salt mixture (3.7%), and dextrin (58.5%). The animals failed to grow. In a subsequent period rapid growth took place when 3 per cent of untreated germ was included in the diet. All the antineuritic substance had been practically destroyed by heating with alkali.

In another set of experiments the effect of glucose, with and without wheat germ extracts, was studied. The results were not always consistent, but it was evident that glucose had a profound stimulating effect. In one case greater growth was secured by adding to 10 cc.

[62] **Sousa and McCollum, J. Biol. Chem. 44, 113, 1920.**

of the control solution 1 cc. of 10 per cent glucose solution than was obtained by addition of 0.1 cc. of 75 per cent alcoholic extract of wheat germ without additional glucose. No modifications which were introduced were successful in inducing concordant results. It seems impossible to avoid irregularities due to the introduction of injured cells or cells of low reproduction power into the test specimens.

In a set of experiments various extracts so treated (with heat under pressure in presence of sodium bicarbonate) that they were no longer capable of producing growth in rats were found to be still very effective in stimulating growth of yeast. Among these were extracts of fresh beef made by boiling the latter with water after it had been treated with 2 per cent sodium bicarbonate, moistened and allowed to stand for three hours, then autoclaved for one hour at 15 lbs. pressure. Extract of rolled oats similarly treated was also used. The oats so treated were fed to young rats in a diet of rolled oats, gelatin, casein, salt, butter-fat and carbonate of lime. The animals failed to grow. After 41 days 3 per cent of untreated wheat germ was added to the diet to furnish the antineuritic substance. The animals responded at once with rapid growth. 40 per cent of untreated oats furnished an abundance of antineuritic *B*, for growth. The treatment with alkali had, therefore, destroyed its content of this substance while leaving the yeast growth-stimulating power apparently unimpaired. Similar results were obtained with extracts of meat which had been digested either with alkali or acid. **Sousa** and **McCollum** state that so far as their knowledge permits them to judge these digested meat extracts could serve only as a source of amino acids since the treatment was sufficient to reduce the proteins practically to the amino acid state, and, since muscle tissue is, even in the fresh condition very poor in the antineuritic, *B*, factor, the same meat after hydrolysis with strong sulphuric acid would scarcely contain a trace of it. Yet these meat extracts exerted a strong influence on the rate of development of yeast, approximately doubling the number of cells counted after 20 hours incubation.

If, then, glucose and amino acids can exert such stimulation on the growth of yeast it would seem to be conclusively demonstrated that the use of yeast as a test organism for determining the presence or absence of the antineuritic dietary factor is complicated by so many disturbing factors as to make it of little if any value.

Confirmatory evidence has been obtained by **Eddy, Heft, Stevenson** and **Johnson**[63] that the yeast test is not a quantitative measure

[63] **Eddy, Heft, Stevenson** and **Johnson,** J. Biol. Chem. 47, 249, 1921; J. S. C. I. 1921, 713 A.

of the content of vitamin *B*. The presence of *B*, however, possibly is one of the factors causing the stimulation of the growth of yeast by vegetable extracts.

Moreover, evidence has been accumulated which appears to demonstrate conclusively that yeast can synthesize the vitamin *B* which it requires. **Fulmer, Nelson** and **Sherwood**[64] found that yeast would grow for months in a vitamin-free medium, at two-thirds the rate manifested in wort. Alcoholic extract of alfalfa stimulated growth, but heating the extract with alkali did not destroy this effect, and alcoholic extracted malt gave results like untreated malt. The growth was markedly influenced by the concentration of the ammonium salt used as nutrient, and by the presence of colloidal substances. They concluded that it is unwarrantable to state that any one substance is indispensable until the best synthetic medium for yeast has been developed. A study of the effect of alcoholic extracts of wheat embryo and lucerne led them to the belief that the growth-stimulant present in these extracts was not identical with vitamin *B*.[65]

MacDonald and **McCollum**[66] grew yeast in nutrient solutions free from vitamin, and found that a pure culture grew as well at the end of a series of 15 consecutive seedings in this solution as it did earlier, and concluded that the yeast plant must either be able to grow without vitamin or else to synthesize this factor in sufficient quantity for its own use. The latter assumption finds confirmation in the work of **Nelson, Fulmer** and **Cessna**,[67] who made 180 consecutive seedings of yeast by adding 1 c. c. to 50 c. c. of a solution containing only carefully purified salts and sucrose. The concentration of the original constituents in the final solution was only 1×50^{-180}, yet it contained enough vitamin *B* to restore rats, fed on a vitamin-poor diet, to normal growth.

It seems highly probable that the growth-stimulant of the American investigators is identical with the "bios" of **Wildiers, Amand** and **Devloo**,[68] but it is as yet impossible to say whether this is the same as vitamin *B* or distinct from it. **Frankel** and **Schwarz**[69] endeavored to isolate the accelerating substance from rice and yeast sediment, but the attempt was unsuccessful.

[64] **Fulmer, Nelson** and **Sherwood**, J. Am. Chem. Soc. **43**, 186; 191, 1921.

[65] See also **Emmett** and **Stockholm**, J. Biol. Chem. **43**, 287, 1920.

[66] **MacDonald** and **McCollum**, J. Biol. Chem. **45**, 307; **46**, 525, 1921.

[67] **Nelson, Fulmer** and **Cessna**, J. Biol. Chem. **46**, 77, 1921.

[68] **Wildiers**, La Cellule, **18**, 313, 1901; **Amand**, *Ib*. **21**, 329, 1904; **Devloo**, *Ib*. **23**, 361, 1906; **Ide**, Centr. Bakt. Abt. 2, 1907; **18**, 193; J. Biol. Chem. **46**, 521, 1921.

[69] **Frankel** and **Schwarz**, Bioch. Z. **112**, 203, 1920.

In this connection the experiments of **Schweizer** [70] are interesting. The action of various fractions of autolysed yeast upon living yeast suspended in pure sucrose solution was measured by the amount of carbon dioxide evolved. The fractions used in the first series of experiments were the alcohol-soluble and alcohol-insoluble portions, and in the second series the cell walls and the cell contents. In both series the separate action of the fractions was small compared with that of autolysed yeast itself. In each case the two fractions when reunited appeared to have the same effect as the original yeast.

Emmett [71] calls attention to the variation in methods employed by different observers in the study of vitamins and recommends that standardized methods be adopted; his suggestions are:

1. There should be a strict uniformity in the basal diets. Thus, in the case of the growing rat, we should agree upon a basal diet that is complete for normal growth and reproduction; upon one deficient only in the water-soluble *B* vitamin, and upon one deficient only in the fat-soluble vitamin or vitamins. And in the case of the guinea pig, we should have a complete basal diet and one deficient in the water-soluble *C*. In these diets, it should be understood that the percentage of protein, carbohydrate, fat and mineral salts, and the kind and quality of these nutrients should be the same throughout, for the particular purpose intended. That is, all laboratories should use exactly the same basal diets. It would then be possible always to employ these rations for the normal and pathological control groups and then to compare with these control findings, the results obtained from any other combination of nutrients that seemed desirable in connection with a particular phase of a vitamin project that one might have under way. That is, if the control data obtained from these basal diets were always reported along with the experimental results, it would be easier to interpret and compare the conclusions from different workers.

2. In the preparation of these basal diets, definite methods should be outlined, with respect to detailing just how to purify the various constituents used — the proteins, carbohydrates, fats, and mineral salts. Besides, exact methods should be given for preparing the vitamin extracts that are to be incorporated. Then again, it should be stated clearly how and in what order these ingredients are to be combined; for unless this is done the texture of the ration will often vary considerably, and thereby an unintentional error will be introduced which might affect the food consumption, and this in turn might influence the trend of the weight curves, etc.

3. It is equally important that there be some definite statements made with respect to the experimental animals. In the case of rats and guinea pigs, the age, weight, sex, and exact physical conditions should be carefully observed and stated. It is known, for example, that, for the best comparisons, the animals should be of about the same age and weight at the start, and that younger rats are preferable to older ones. In Emmett's work where he used some 2,000 pigeons, he found that it was necessary to consider breed, previous feeding, age,

[70] **Schweizer,** Bull. Assoc. Chim. Sucr. 38, 304, 1921; J. S. C. I. July 30, 1921, 524A.

[71] J. Ind. Eng. Chem. 1921, 1104.

body conditions, weight, season of the year, etc., in order to obtain the best results in testing for the antineuritic vitamin. Also, in the case of handling and taking care of the animals, it is essential to bear in mind that one should avoid exposing them to sudden changes in temperature and to undue excitement, and to too much handling. Systematic attention should be given to cleaning and disinfecting the cages, bedding, food cups, and water receptacles. Vermin or lice should be scrupulously excluded. Special care should be given an animal when it becomes appreciably affected by the deficiency disease, and so on. In fact, it is only after considerable experience that one can always be sure the effect of the experimental diet is a bigger factor than some of the other variables that may have been introduced.

4. It is very important to state whether the animals are to be force-fed or not; whether the food in-take is to be accurately recorded or not; whether the rations are to be made frequently or in large enough quantity to last for a considerable time; whether the consistency of the food or ration is appreciably altered when the changes in the diet are made, and, if vitamin extracts are to be used, whether the animals are to be treated during the test period, or fed at this juncture a mixture of the basal diet and the extract.

5. In the feeding, the question arises whether the rations should be tested from the standpoint of a prophylactic or a corrective. That is, should the test be one of preventing the usual decline in weight, etc., that comes about from the absence of the particular vitamin, or should it be one endeavoring to bring about a normal response after the animal has been affected? In many ways, it is often preferable to follow the latter method.

6. It is very important that the tests should be run long enough to be conclusive. Emmett reports instances in the case of the rat, where the pathological animals did not respond quickly when put upon a curative diet. Sometimes they did not manifest any signs of improvement for almost a week, when they began to show the effects of the new diet. Here it is not a case of the vitamin being absent but simply a question of the animal not eating the new ration at first. When they do begin to take the food, the effect of the treatment will become very evident. However, the precaution should be taken to make sure that any gains in weight are due simply to a change in the physical nature of the food, rendering it possibly more palatable. Noting the physical condition of the animal and continuing the test long enough will settle this point. Where it is possible, as in using extracts, it is generally much better to keep the basal diet the same throughout and give the animals definite doses of the extracts. In this way, one can make sure that the material has been consumed. This, of course, cannot be done where one wishes to test a food in its entirety.

CHAPTER III

DIETARY FACTORS OTHER THAN VITAMINS

It is a self-evident fact that conclusions concerning any one factor in a diet will be reliable only if the ration fed is entirely satisfactory in every respect save the particular factor under investigation. Much of the early work on vitamins was unsatisfactory because of the faulty character of the basal diet, the fault being due to lack of adequate knowledge rather than to any carelessness on the part of the investigators. In recent years great advances have been made in our knowledge of the quantitative composition of the natural foodstuffs and of the requirements of young and adult animals, especially with regard to amino acids and mineral salts.

McCollum is responsible for the introduction of what he has designated "biological analysis" of foods, that is, a systematic series of feeding experiments in which first one dietary factor at a time, and then if necessary combinations of two or more factors, are altered in order to ascertain the true function of each. This method is very well illustrated by the experiments of **McCollum** and **Davis** on the dietary value of the wheat grain.[1]

It was reasoned that, since all the dietary essentials, except possibly the one which is not present in vegetable fats, are certainly present in the wheat kernel, the faults in the latter must depend upon a lack of the unknown substance contained in butter-fat, or on the quantity of some one or more of the well-recognized constituents of the diet. It seemed possible to discover by means of a systematic series of feeding experiments in which the quality of the seed should be improved with respect to one dietary factor at a time, which factor was interfering with growth. Accordingly wheat was fed in the following combinations, and with the results noted:

(1) Wheat alone ....no growth, short life
(2) Wheat plus purified protein ....no growth, short life
(3) Wheat plus a salt mixture which gave it a mineral content similar to that of milk ....very little growth
(4) Wheat plus a growth promoting fat (butter-fat) ....no growth

From these results it seemed apparent that either their working hypothesis regarding the factors which are necessary in an adequate diet, must be wrong, or there must be more than a single dietary factor of poor quality, and jointly

[1] **McCollum,** "Newer Knowledge of Nutrition," 1919, p. 20.

responsible for the poor nutrition of the animals. In order to test this theory another series of feeding experiments was carried out in which wheat was fed, supplemented with two purified food additions.

(5) Wheat plus protein, plus the salt mixture..........Good growth for a time. Few or no young. Short life.

(6) Wheat plus protein, plus a growth-promoting fat (butter-fat)....No growth. Short life.

(7) Wheat plus the salt mixture, plus the growth-promoting fat (butter-fat) ....Fair growth for a time. Few or no young. Short life.

Following this method **McCollum** and his co-workers have made an exhaustive study of a great many typical foodstuffs and have arrived at results of immense practical value, which must be taken into consideration in all feeding experiments in which natural foodstuffs form part of the diet. In so far as these deal with factors other than vitamins they are briefly summarized here for reference.

Experiments with swine [2] showed that there was very little difference in the nutritive value of the proteins contained in wheat, oat and corn kernels, and at a single experiment in which the three grains were fed together in equivalent amounts there appeared to be little advantage through the supplementary action of the proteins from one source on those from another.

Hart and **McCollum** [3] showed that when swine are restricted to corn meal and gluten feed little or no growth can be secured, but with an addition of salts, making the entire ash content of the ration very similar in quality to that of milk, growth approximating normal was secured to at least 275 pounds. On an exclusive diet of wheat kernel, growth was again limited, both with swine and rats. Correcting the mineral content alone induced a certain amount of growth, but the benefit was only temporary. Supplementing the wheat kernel with salts and butter-fat caused the animals to remain vigorous and strong for a very much longer period, and induced improved growth in both species, although not up to normal. When a further addition of casein to the extent of 2.5 per cent of the ration was made a normal curve of growth was secured with both swine and rats. A diet of mixed grains and distilled water did not allow normal growth in swine.

In a study of the influence of protein intake on growth, **McCollum** and **Davis** [4] found that there is a progressive increase in the rate of growth on rations derived from milk as the plane of protein intake is raised between three and eight per cent of the diet, and that, during

[2] McCollum, J. Biol. Chem. 19, 323, 1914.

[3] Hart and McCollum, J. Biol. Chem. 19, 373.

[4] McCollum and Davis, J. Biol. Chem. 20, 415, 1915.

six weeks at least, a ration carrying but four per cent of protein from wheat embryo compares favorably with a similar plane of protein intake derived from milk powder, and is somewhat better than six per cent of protein from the entire kernel, on which rats may grow at about half normal rate.

An investigation on the influence of the mineral content of the ration on growth and reproduction [5] led to the conclusions that provided the other factors in the ration are adequate, young rats can grow normally and remain in apparent good health on rations whose base content varies widely in amount (from 0.2 to 8 per cent); and that high acidity is not necessarily injurious.

McCollum and **Davis** [6] studied the dietary deficiencies of rice and showed that polished rice must be supplemented with protein and salts, as well as with vitamins *A* and *B*, in order to make it a complete ration.

McCollum, Simmonds, and **Pitz** [7] investigated the nutritive value of the wheat embryo (commercial preparation) and concluded that it contains qualitatively all the factors essential for the promotion of growth and well-being in an animal but in amounts which are not all satisfactory, the mineral content in particular requiring modification before growth can proceed at all. The character of the proteins appeared to be unusually excellent, an amount equal to 10 per cent of the ration being adequate for growth at the maximum rate. They state that the wheat germ contains an ether-soluble substance which is toxic to animals (rats in this case), which would seem to be confirmed by the observations of **Hart, Miller** and **McCollum** [8] that with a large mass of wheat in the ration of swine, toxcity will follow even in the presence of all the recognized factors for growth.[9] Only in the presence of very liberal quantities of all these factors can the effect of the toxicity be overcome, no one factor being able to act as a complete corrective. The pathological conditions produced by this toxicity closely resemble those due to a deficient salt supply. This alleged toxicity of the wheat germ is however disputed by **Osborne** and **Mendel**, who found no evidence [10] of it in their exhaustive study

[5] **McCollum** and **Davis**, J. Biol. Chem. 21, 615.

[6] **McCollum** and **Davis**, J. Biol. Chem. 23, 181, 1915.

[7] **McCollum, Simmonds** and **Pitz**, J. Biol. Chem. 25, 105.

[8] **Hart, Miller** and **McCollum**, J. Biol. Chem. 25, 249.

[9] See also, **Hart, McCollum, Steenbock** and **Humphrey**, Proc. Nat. Acad. Sc. 1917, 3, 374, J. Agric. Res. 1917, 10, 175; **Hart, Steenbock** and **Humphrey**, Univ. Wisc. Agric. Exp. Sta. Bull. 287, 1918; **McCollum, Simmonds** and **Pitz**, J. Biol. Chem. 28, 211.

[10] **Osborne** and **Mendel**, J. Biol. Chem. 37, 557.

of the nutritive value of the wheat kernel and its milling products, and similar conclusions are reached by **Voegtlin** and **Meyers.**[11]

In a second paper on the effects of wheat feeding, **McCollum, Simmonds** and **Pitz**[12] report that they were unable to make up a ration containing wheat proteins only which was adequate for rearing the young, even though they varied the protein content from 6.5 to 47.98 per cent. Growth approximated normal over a wide range of protein content, and the injurious effects of the ration showed in the reproduction records only. Marked improvements resulted from the addition of 10 per cent of casein to a ration containing 36.33 per cent of protein from wheat.

McCollum, Simmonds and **Pitz**[13] in experiments with rats found that the addition of purified protein and salts, or of butter fat and salts to maize kernel fails to induce physiological well-being throughout the life of an animal, and the addition of protein and butter fat without salts is even poorer.

The oat kernel was found to contain proteins of poorer quality for growth[14] than either maize or wheat[15] the deficiency being supplemented more satisfactorily by gelatin than by casein. The whole oat kernel, with the hulls removed by coarse grinding and jamming, fails to induce growth; addition of protein, salt mixture, and butterfat being necessary before anything approaching normal growth to maturity can be secured, and even with these supplements reproduction is a complete failure. Moreover oats produce feces of a pasty character which makes their elimination difficult and probably tends to debilitate the animal. The protein of the common white bean *(Phaseolus vulgaris)* is of unusually poor biologic value,[16] and the mineral content also needs supplementing as would appear to be the case with all seeds. Moreover the bean appears to exert an injurious effect on the rat when fed to high level in the ration, possibly due to the introduction into the digestive tract of large quantities of the indigestible but readily fermentable hemicelluloses.

Curiously, peas (dry split peas) appear to be more satisfactory than beans as a food for rats,[17] inasmuch as feeding liberal amounts

[11] **Voegtlin** and **Myers,** Public Health Reps. 1918, 33, 843.

[12] **McCollum, Simmonds** and **Pitz,** J. Biol. Chem. 28, 211.

[13] **McCollum, Simmonds** and **Pitz,** J. Biol. Chem. 28, 153, 1916.

[14] Cf. maintenance value as given by **McCollum** and **Simmonds,** J. Biol. Chem. 32, 347.

[15] **McCollum, Simmonds** and **Pitz,** J. Biol. Chem. 29, 341.

[16] **McCollum, Simmonds** and **Pitz,** J. Biol. Chem. 29, 521; **Osborne** and **Mendel,** J. physiol. Chem. 80, 307, 1912. See also **Johns** and **Finks,** Am. J. Physiol. 56, 205, 208, 1921.

[17] **McCollum, Simmonds** and **Parsons,** J. Biol. Chem. 37, 287.

of peas supplemented with casein, calcium and sodium chloride over considerable periods of time permitted growth to maturity and even reproduction, although the young could not be reared. Pea protein is however very unsatisfactory in character.[17a] A diet containing 20 per cent of pea protein and adequate in other respects gave a rate of growth less good than has been observed with diets containing but half as much protein derived from one of the cereal grains. Even when supplemented with protein and vitamin *A*, peas to the extent of 85 per cent of the food mixture cannot support growth, because of the shortage of certain inorganic elements, addition of calcium, sodium, and chlorine being necessary in order to make the diet satisfactory for the support of growth at the optimum rate. Two experiments were made in one of which rats fed on liberal amounts of peas, supplemented with protein, *A*, and salt mixture, failed to grow satisfactorily, while in the other test, rats fed with a diet supplemented with essentially the same purified additions, but containing but half as much peas, were much better nourished, and produced what is regarded as the normal number of young, although none were successfully weaned. This suggests that there is present in peas some substance or substances which prove injurious when taken in large amounts. The toxicity, however, if there be any, is slight, and only manifests itself when diets extremely rich in peas are persisted in over a long period.

In order to ascertain the biological value of mixtures of cereal and legume seeds, **McCollum** and **Simmonds**[18] experimented with mixtures of maize and beans as a diet for rats. The mixture, as was to be expected, proved deficient in mineral content and *A* and in protein, The protein in what was apparently the optimum mixture of 80 per cent maize and 20 per cent beans had just about one-half the biological value for growth that the total protein mixture in milk possesses.

A further study of the values of some seed proteins for maintenance was made by **McCollum** and **Simmonds.**[19] Since it has been repeatedly shown that seeds in general are lacking in three dietary factors, mineral content, *A* vitamin, and protein, it follows that if a particular seed is fed supplemented with both salts and butter-fat, the limiting factor is the quality of the protein. In order to determine the relative value of the total protein the following method was employed:

(a) The seed supplemented with the necessary salt and butter

[17a] See **Sure,** J. Biol. Chem. 46, 443, 1921.

[18] **McCollum** and **Simmonds,** J. Biol. Chem. 32, 29.

[19] **McCollum** and **Simmonds,** J. Biol. Chem. 32, 347.

fat is fed alone; (b) a pure carbohydrate is added to produce a low protein mixture; (c) the supplemented seed is fed with a protein preparation from the same seed so as to raise the plane of protein intake to higher levels. The results show the relative value of the total protein of the seed as compared with that of other seeds. It was found that with rats six per cent of wheat or maize proteins just suffice to maintain the animals in body weight, while four per cent of oat proteins maintained them in distinctly better condition as judged by their appearance than did six per cent of wheat and maize proteins. Flaxseed proteins appear to have a still lower biological value when fed as the sole source of protein. It requires about eight per cent of flaxseed proteins in the food to maintain a full grown rat. Millet seed proteins possess a somewhat higher value for maintenance. Pea and bean proteins possess about the same biological values when fed alone, both being inferior to either wheat or maize. The value of the bean proteins is enhanced by the addition of oat proteins while that of the pea is not. Contrary to the conclusions of **Thomas**,[20] the proteins of polished rice appear to be of about the same quality as those of wheat and maize.

When mixtures of seeds are fed there is some improvement in the biological values of the proteins over those derived from a single seed. **McCollum and Simmonds**[21] aver that in certain cases the value of such mixtures doubtless are high, but with simple mixtures of the cereal grains they were not able to demonstrate a high degree of efficiency as a source of protein in any case.

Since young rats are able to grow at approximately half the normal rate on food mixtures in which protein from maize, rye or barley are present to the extent of nine per cent of the food mixture, and which are otherwise entirely adequate, **McCollum, Simmonds and Parsons**[22] carried on a series of experiments in which the food contained nine per cent of protein derived from two seeds, one furnishing two-thirds, and the other one-third of the total protein, together with adequate amounts of the other necessary ingredients, and compared the growth obtained with that produced by an equivalent amount of protein from a single seed. Of the single seeds, wheat gave the best results, rolled oats the poorest, but a ration containing eight per cent of protein from milk powder was far superior to one containing nine per cent of wheat protein. For the most part the proteins from the mixed grains seemed to offer little advantage over those from

[20] Thomas, Arch. Physiol. 1909, 219.

[21] McCollum and Simmonds, J. Biol. Chem. 33, 303.

[22] McCollum, Simmonds and Parsons, J. Biol. Chem. 37, 155.

a single seed for growth, the most striking exception being a mixture of rye and flaxseed in which rye furnished protein equal to six per cent and flaxseed half as much, which, for growth, appeared to be almost if not quite equal to proteins of milk. A combination in which two-thirds of the protein came from peas and one-third from millet also gave good growth and reproduction, and millet proved to have some supplementary value when combined with rolled oats also.

In no case can the necessary mineral content of the diet be satisfactorily supplied from seeds alone.[23] Even with mixtures of two to five seeds,[24] additional sodium, chlorine, and calcium must be supplied before any growth can be obtained.

The value of the leaf as a supplement to the deficiencies of seed rations was studied by **McCollum, Simmonds** and **Pitz,**[25] who found that a mixture of sixty per cent seed and forty per cent alfalfa flour gave much more satisfactory growth than can any seed diet unless extensively supplemented. Sixty per cent of rolled oats and forty per cent alfalfa leaf induces nearly normal growth to adult size in the rat. In this connection **McCollum**[26] says:

> "The leaf proves to be a very different thing from the seed from the dietary standpoint. The dry leaf usually contains from three to five times as much total ash constituents as does the seed, and is always especially rich in just those elements in which the seed is poorest, viz., calcium, sodium, and chlorine. It follows therefore that the leaf supplements the inorganic deficiencies of the seed. The leaf, in most cases, contains much more of the dietary essential, fat-soluble *A*, than is found in any seed, so that combinations of leaf and seed prove more satisfactory for the nutrition of an animal than do mixtures of seeds alone. The leaf contains protein and amino-acids which result from digestion of proteins as does the seed. The amount varies from eight per cent of protein (nitrogen multiplied by 6.25) in such fleshy leaves as the cabbage, after drying, to more than fifteen per cent in the dry alfalfa or clover leaf. The seeds vary in their content of protein from about ten to twenty-five per cent. The leaf proteins appear, from the data available, to supplement and enhance in some degree the value of the seed proteins with which they are combined. The leaf supplements, therefore, all the nutritive deficiencies of the seed, but not necessarily in a highly satisfactory manner."

A study of the dietary properties of tubers, as represented by the potato, showed[27] that dietary props of potato closely resemble those of cereal grains. The first limiting factor for growth is a relative shortage of calcium, sodium and chlorine, as has been found

[23] **McCollum, Simmonds** and **Pitz,** J. Biol. Chem. 30, 14.
[24] **McCollum** and **Simmonds,** *Ibid.* 33, 303.
[25] **McCollum, Simmonds** and **Pitz,** J. Biol. Chem. 30, 13.
[26] **McCollum,** "Newer Knowledge of Nutrition," 1919, p. 43.
[27] **McCollum, Simmonds** and **Parsons,** J. Biol. Chem. 36, 197.

to be the case with seeds thus far examined. The content of *A* is too low for promotion of nutrition at optimum, and the biological value of nitrogen yielded seems to be of about the same order as that of cereal grains. The results on the value of the potato nitrogen for growth place it in a very different light from those reported by other investigators who have observed its value for maintenance of nitrogen equilibrium in the adult, all ascribing to potato nitrogen a biological value considerably greater than that found by McCollum and his associates for any of the protein mixtures contained in the more important seeds employed as human foodstuffs.[28]

Cooked dried potato can be supplemented by the addition of purified protein, sodium chloride, calcium carbonate, and a fat containing *A*, so as to be satisfactory support of growth at about normal rate, and to induce reproduction and rearing of the young. The mortality of the young was high and the fertility of adults decidedly below normal. Young from these rats have grown at the normal rate for about three months after weaning, when confined to the diet to which their parents have been confined throughout life, and one gave birth to three young.

The beet appears to resemble the potato in general nutritive character. Referring to this McCollum[29] says:

"The fleshy roots of the potato and the sweet potato have an inorganic content which resembles that of the seed in a general way, so that an inspection of the analytical data relating to the composition of the ash of the seeds, tubers, and roots, gave no promise that the combination in diets of seeds with either of the latter classes of foodstuffs would correct the inorganic deficiencies of the former. Feeding experiments in which a seed and a tuber were combined, and so supplemented with purified protein, and fat-soluble *A* (in butter fat), that all the deficiencies of the mixture, except the inorganic, were made good, have shown that in the combinations of each of the more important seeds with the potato, the resulting mineral supply, which is derived solely from the natural foods themselves, is not of a character suitable for the support of growth. The content of the elements, calcium, sodium and chlorine, must be augmented by greater amounts before such food mixtures are complete with respect to their mineral content. No studies have as yet been made to determine the biological value of the nitrogen of the tubers other than the potato, and none at all of the edible roots.

"From the results of systematic feeding trials with mixtures of seeds alone and the same with single and multiple purified food additions, and the same type of experiment using certain of the tubers and root foods in place of the seeds, it is shown that all these classes of foodstuffs resemble one another in

[28] Hindhede, Ugesk. Laeger, 79, Nos. 13, 14, 15, 1917; J. Am. Med. Asso. 68, 1880, 1917; Thomas, Arch. Physiol. 1909, 219; Rose and Cooper, J. Biol. Chem. 30, 201.

[29] "Newer Knowledge of Nutrition," 1919, p. 48.

all respects except in the high content of water in the tubers and roots. In the dry state, they are all much like the seeds, but there is one minor difference which should be mentioned. The most important difference lies in the character of the nitrogen compounds. In the seeds the nitrogen is almost all contained in the form of true protein. In the tubers and edible roots most of it is in the form of much simpler compounds, a part being the same amino-acids which are derived from proteins on digestion."

Running parallel with **McCollum's** experiments in biological analysis is an equally interesting and valuable series of papers by **Osborne** and **Mendel**, besides various communications from other authors.

Osborne and **Mendel** [30] demonstrated that the proteins are not all equally adequate in nutrition, but that their biological efficiency depends on the amino acids present. They showed that a supply of tryptophane in the food is necessary in order to maintain an animal without loss, and that in addition lysine must be present to secure growth. The rate of growth appears to be limited by the proportions of these amino acids furnished.[31]

Proteins from one source which contain an insufficient supply of the essential amino acids may be supplemented by combining with proteins from other sources which have a surplus of those acids which the first lacks. Thus the lactalbumin of milk supplements the inadequate protein of corn most satisfactorily,[32] while gelatin is a better supplement for oat protein.[33]

Studying the comparative nutritive value of different proteins for growth by feeding exactly equivalent amounts of energy in the form of foods with unlike proportions of the individual proteins, and observing the minima for growth and the limits of intake for maintenance as shown by failure to make proper gains or to maintain weight, **Osborne** and **Mendel** conclude that the economy of the different proteins as nutriments in growth appears to be closely bound up with the constitution of their amino acids. Normal growth is attained on a diet containing twelve per cent of casein, but the addition of isolated cystine to a diet containing only nine per cent of casein renders it equally efficient, indicating that the proportion of this acid in casein is below the optimum for growth. Lactalbumin appears to be the most efficient of all proteins for growth.[34] Rats could be maintained without loss of weight with a smaller intake of this

[30] **Osborne and Mendel**, Carnegie Publication 156, 1911, J. Biol. Chem. 12, 473, 13, 233.

[31] J. Biol. Chem. 17, 325; 25, 1.

[32] J. Biol. Chem. 18, 1; 26, 293; 29, 69; 44, 1.

[33] J. Biol. Chem. 34, 521.

[34] J. Biol. Chem. 20, 121.

than of any other protein studied,[35] maintenance being frequently secured with as little as five per cent of lactalbumin in the diet as the sole source of protein. When corn or corn meal formed the chief constituent of the diet of white rats it was found necessary to supplement this with a protein concentrate rich in tryptophane and lysine in order to secure the best results.[36]

An attempt to determine the relative values of the proteins in the various cereal grains proved difficult. The general conclusion was drawn that the total proteins of rice and barley, in contrast to those of corn and oats, when furnished in diets containing 16 to 17 per cent of protein, supply enough of all the amino acids essential for growth.

Later [37] there seemed to be some indication that oats can actually furnish all the essential nitrogenous units if the intake of food and its concentration of protein are adequate, but as a rule, rats failed to eat the oat foods very heavily. The value of rye proteins is questionable, and wheat appears to be unsatisfactory as an exclusive source of protein,[38] since, although the first generation could be raised to full adult size on a diet containing ten per cent of protein from wheat, the second generation on this food failed to grow with normal vigor.[39] It appears that the proteins of the wheat endosperm are adequate for maintaining adults, but that they are inadequate for growth.[40] Additions of meat, milk, or eggs to wheat flour so greatly enhance the value of the protein for growth that a great economy in consumption of protein results.[41]

A method of expressing the growth-promoting value of proteins numerically was worked out by **Osborne, Mendel** and **Ferry** [42] who found that the maximum efficiency of the different proteins or of mixtures of them can be expressed with considerable accuracy in terms of gain of body weight per gram of protein eaten, provided the rate of growth is fairly rapid. With a diet containing 7.9 per cent of lactalbumin the maximum gain of weight per gram of protein eaten was three grams, which is approximately the maximum growth-promoting capacity of lactalbumin. On a similar diet with casein as a sole source of protein the greatest gain per gram of protein was 2.25 grams when the diet contained 12 per cent of casein.

[35] J. Biol. Chem. 22, 241; 26, 1.

[36] Proc. Am. Physiol. Soc., Am. J. Physiol. 40, 147.

[37] J. Biol. Chem. 41, 275.

[38] **Osborne** and **Mendel**, l. c.; **McCollum, Simmonds** and **Parsons**, J. Biol. Chem. 37, 155.

[39] J. Biol. Chem. 37, 557.

[40] J. Biol. Chem. 41, 290.

[41] J. Biol. Chem. 34, 521.

[42] **Osborne, Mendel** and **Ferry**, J. Biol. Chem. 37, 223.

For further reference to factors other than vitamins in the diet see also:

McCollum (Am. J. Physiol. 1911, 29, 215). The nature of the repair processes in protein metabolism.

McCollum and **Davis** (*Ibid.* 1915, 20, 415). The influence of protein intake on growth.

Rohmann (Bioch. Zeitschr. 1912, 39, 507). Artificial nutrition.

Abderhalden (Zeitschr. physiol. Chem. 1912, 77, 22). Feeding investigations with completely digested nutrients. (*Ibid.* 1913, 83, 444). Synthetic powers of the organism of the dog. (*Ibid.* 1915, 96, 1). Nitrogenous metabolism.

Totani (Bioch. J. 1916, 10, 382). Feeding experiments with a dietary in which tyrosine is reduced to a minimum.

Mitchell (J. Biol. Chem. 1916, 26, 231). Feeding experiments on the substitution of protein by definite mixtures of isolated amino acids.

Ackroyd and **Hopkins** (Bioch. J. 1916, 10, 551). Feeding experiments with deficiencies in the amino acid supply.

Hopkins (J. Chem. Soc. 1916, 629). Newer standpoints in the study of nutrition.

Buckner, Nollau, and **Kastle** (Am. J. Physiol. 1915, 39, 162). Feeding young chicks on grain mixtures of high and low lysine content.

Hogan (J. Biol. Chem. 1916, 27, 193). Nutritive properties of maize. *Ibid.* 1917, 20, 485). Maize as a source of protein and ash for growing animals.

Sherman, Wheeler, and **Yates** (*Ibid.* 1918, 34, 383). Experiments on the nutritive value of maize protein.

Sherman and **Winters** (*Ibid.* 1918, 35, 301). Efficiency of maize protein in adult human nutrition.

Hart, Halpin and **Steenbock** (*Ibid.* 1917, 31, 415). Behavior of chicks restricted to wheat or maize kernel.

Morgan and **Beger** (Zeitsch. physiol. Chem. 1915, 94, 324). Injurious effect of an exclusive oat diet attributable to an acid intoxication.

Sherman, Winters and **Phillips** (J. Biol. Chem. 1919, 39, 53). Efficiency of oat protein in adult human nutrition.

Steenbock, Kent and **Gross** (*Ibid.* 1918, 35, 61). Dietary qualities of barley.

Hart and **Steenbock** (*Ibid.* 1919, 39, 209). Maintenance and reproduction on grains and grain products as the sole dietary.

Daniels and **Loughlin** (*Ibid.* 1918, 33, 295). Feeding experiments with peanuts.

Johns and **Finks** (*Ibid.* 1920, 42, 569). Nutritive value of peanut flour as a supplement to wheat flour.

Richardson and **Green** (*Ibid.* 1916, 25, 307; 1917, 30, 243; 31, 379). Nutrition investigations on cottonseed meal.

Wells and **Ewing** (*Ibid.* 1916, 27, 15). Cottonseed meal as an incomplete food.

Daniels and **Nichols** (*Ibid.* 1917, 32, 91). Nutritive value of the soy bean.

Osborne and **Mendel** (*Ibid.* 1917, 32, 369). Use of the soy bean as food.

Rose and **Cooper** (*Ibid.* 1917, 30, 201). The biological efficiency of potato nitrogen.

Daniels and **Rich** (*Ibid.* 36, 27, 1918). The rôle of inorganic sulphates in nutrition.

Plimmer (J. S. C. I., 40, 227R, 1921). The relative value of the proteins in nutrition.

Physiology of Protein Metabolism, Cathcart, London, 1921.

While the basal diets used in vitamin studies vary with every investigator, almost with each investigation, certain general principles are followed. The protein of the basal ration is very commonly furnished by casein (or caseinogen, see p. 263), and the carbohydrate by either dextrin or starch, the lactose used in the early experiments having been found difficult to purify. **Drummond**[43] recommends the use of rice starch, which, in the crude state, is almost entirely devoid of vitamin and may therefore be employed without any lengthy extraction. In order to remove any possible traces of vitamin from the casein **Funk**[44] resorted to extraction with hot alcohol. This method was objected to by **McCollum** and **Davis** on the ground that the high temperature might affect the casein unfavorably.[45] Since experiments had shown that casein suffers deterioration on prolonged heating **McCollum** and **Davis** thought it unwise finally to extract this material for a long period with boiling alcohol, as **Funk** and **McCollum**[46] had done in order to remove all traces of unknown accessory substances. Through this treatment the value of the casein may be decidedly reduced.

The method adopted was the following: Casein purified by twice repeated precipitation was washed, dried, and ground. It was then placed in a large jar having an outlet at the bottom which was closed with a plug of cheese-cloth loose enough to permit a slow passage of water through it. The jar was filled with water acidified with acetic acid. When it had nearly all drained off the jar was again filled. The casein was frequently stirred to prevent its forming a compact mass. This washing was continued during seven or eight days, the last twenty-four hours washing being with distilled water. The product thus obtained was dried and ground. It was very poor in ash, 10-gram samples yielding but a trace of calcium. By this treatment practically all the water soluble constituents were dialyzed out of the granules. With casein prepared in this way combined with dextrin, butter-fat, and salts no appreciable growth was obtained even during the first month.

Funk and **McCallum**[47] tested casein prepared by the method just given and compared it with that prepared by their own method, with identical results. They suggest that heated casein may possibly have its value reduced through loss of *C*, but this is easily made good by addition of orange juice to the diet.

Osborne and **Mendel** at one time[48] believed that casein prepared

[43] **Drummond** and **Coward**, Bioch. J. 14, 661, 1920.

[44] **Funk** and **Macallum**, Zeitschr. f. physiol. Chem. 92, 17; J. Biol. Chem. 23, 413, 1916.

[45] **McCollum** and **Davis**, J. Biol. Chem. 23, 233, 1915.

[46] **Funk** and **Macallum**, Zeitschr. f. physiol. Chem. 92, 17.

[47] **Funk** and **Macallum**, J. Biol. Chem. 27, 60, 1917.

[48] **Osborne** and **Mendel**, J. Biol. Chem. 34, 156, 1918.

by ordinary methods was entirely satisfactory, without further purification, for use in experiments with vitamin *A*. Subsequent experience however has led them to modify their views somewhat. In a later publication [49] they discuss the allegation that the ability of animals to grow for some time in the supposed absence of fat-soluble vitamins is due to "exceptional vitality" of the individuals or to reserve stores of the vitamin in the body. Unless one accepts some indefinite explanation of this sort, it seems necessary, according to Osborne and Mendel, to conclude in the light of their experience, that removal of the fat-soluble vitamin from even purified proteins and carbohydrates is accomplished with far greater difficulty than has been hitherto suspected. An entirely convincing crucial experiment, in which nutritive failure immediately ensues upon the administration of diets fully adequate in every respect except for the presence of fat-soluble vitamin, remains to be made. It is significant that older rats thrive for a longer time than do the younger ones on the same diets nearly if not entirely free from the fat-soluble vitamin. This is in contrast with the well-established fact that at all periods the lack of water-soluble vitamin is speedily manifested. They found that rats were capable of appreciable growth for some time on a diet of casein or edestin, commercial corn starch and dried brewery-yeast (all of which had been purified by boiling three times with absolute alcohol under a reflux condenser for one hour), a salt mixture, and lard (either commercial lard, alcohol-extracted lard, or lard rendered at comparative low temperatures in their own laboratory).

Drummond and Coward [50] state that commercial casein contains relatively large amounts of *A* and that animals may obtain sufficient of this vitamin for prolonged growth from impure casein incorporated in the diet. They recommend the use of casein which has been heated for 24 hours or more to a temperature of 102° C, in shallow dishes, and then subjected to prolonged and continuous extraction with alcohol and ether.

In all tests on vitamin *A* Drummond and Coward [51] recommend the use of a hydrogenated vegetable oil instead of a natural fat, since even those vegetable oils which are supposed to be devoid of *A* are not entirely so. Hydrogenated cottonseed oil they regard as absolutely vitamin-free, however, so far as could be ascertained by numerous tests.

[49] Osborne and Mendel, J. Biol. Chem. 45, 277, 1921.
[50] Drummond and Coward, Bioch. J. 14, 661, 1920.
[51] Drummond and Coward, l. c.

Mineral Salts

It has been frequently demonstrated that it is impossible to secure growth or maintenance if the inorganic constituents of the diet are improperly adjusted.[51a] The most satisfactory mixture of salts to supplement a salt-free diet attained to by **Osborne** and **Mendel** in their early experiments, after many attempts to modify the relative proportions of the differentiations present, was essentially that used by **Rohmann.**[52]

Salt mixture I (Rohmann and Osborne and Mendel)

Calcium phosphate	10.0	grams
Potassium hydrogen phosphate	37.0	"
Sodium chloride	20.0	"
Sodium citrate	15.0	"
Magnesium citrate	8.0	"
Iron citrate	2.0	"
Calcium lactate	8.0	"
	100.0	grams

2.5 per cent of this was incorporated in their salt-free rations.

Having observed the excellent results attained with rats fed on a food mixture of milk powder (60%), starch (16.7%), and lard (23.3%), in which the inorganic salts were obtained entirely from milk, the idea suggested itself that milk freed from fat and protein

[51a] **Scala** (Ann. igiene **29**, 215, 286, 1919; Expt. Sta. Record **43**, 462) advances the hypothesis that deficiency diseases originate in a deficient mineral nutrition, either in the lack of certain acids or bases or of complexes by means of which they are transported. In scurvy the inorganic substance which is lacking is thought to be phosphates of the earth metals, which exist in food materials in combination with organic material in the form of complexes, easily decomposed by heat and by desiccation. The destruction of these complexes tends to bring about a state of acidosis and prevents the transportation of calcium phosphate, etc., to the bones. In beriberi the alkali phosphates are unable to reach the central nervous system through the destruction of the organic complexes containing them and the development of a form of acidosis. The fact that various mineral substances when added to a beriberi-producing diet fail to bring about appreciable benefit is explained on the ground of inability to reproduce the exact complexes found in the original food material.

Loew (Vierteljahrs. ger. Med. **61**, 151, 1921) contends that calcium equilibrium depends on the nature of the diet. A high-fat diet means a low calcium assimilation. A decrease in the alkalinity of the blood means a loss of calcium by excretion. A prolonged under-supply of calcium gives rise to diverse pathological conditions. (See also, **Rubner**, Vierteljahrs. ger. Med. **61**, 155 1921).

[52] Jahresb. f. Thier. Chem. 38, 659, 1909.

might be used with advantage as the source of inorganic salts. Accordingly they prepared a product designated "protein-free milk," as follows: [53]

Perfectly fresh centrifugated milk, nearly free from fat, was precipitated in lots of about 36 liters by diluting with 7 liters of distilled water which contained 164 cc. of concentrated hydrochloric acid. The flocculent precipitate of casein was strained out on cheesecloth, and the very nearly clear solution was filtered through a pulp filter. The filtrate, which at the most was very slightly turbid from suspended fat, was tested carefully by the alternate addition of dilute alkali and acid to determine whether any more casein could be separated from it. The addition of alkali caused a slight precipitate which did not increase on adding more alkali or dissolve on the addition of even relatively large amounts of alkali. This was presumably chiefly calcium phosphate. The addition of acid in no case caused any further precipitation. The filtered milk serum was then heated to boiling for a few minutes and filtered. The filtrate, which was in all cases water clear, was then neutralized to litmus with a dilute solution of sodium hydroxide and evaporated to dryness on a steam bath at a temperature of about 70° C. The product thus obtained formed a friable, pale yellow mass which was easily reduced to a fine powder by grinding in a mill.

With this preparation, as has been already pointed out, they obtained results successful beyond their expectations; results which have been confirmed by later investigations.[54]

Considerable discussion arose as to the amount and effect of the nitrogen remaining in this "protein-free milk." In their original communication these investigators say:

> Several grams of this powder were tested for protein by dissolving in about 30 cc. of water containing a little hydrochloric acid and warming gently. The solution was then saturated with ammonium sulphate. The precipitate, which appeared to consist chiefly of calcium sulphate was separated by centrifugation, dissolved in a little water, and potassium hydrate solution and copper sulphate added. The solution showed no evidence of the biuret reaction until it was saturated with potassium hydroxide and shaken with alcohol. It then separated into two layers, the upper alcoholic layer showing a slight but positive biuret reaction. Millon's reaction tried on portions of two or three grams of the substance did not give a positive reaction. Nitrogen determinations in several lots of the protein-free milk powder thus made showed them to contain 0.66, 0.59, 0.60, 0.72, 0.71, 0.67, 0.75, per cent of nitrogen. Munk [55] states that if the

[53] Osborne and Mendel, Carn. Pub. 156, Pt. 2, p. 80–81.

[54] Wheeler, J. Exper. Zool. 15, 209, 1913; Mitchell and Nelson, J. Biol. Chem. 23, 461.

[55] Munk, Virchow's Archiv. f. path. Anat. 134, 501, 1893.

proteins of milk are precipitated by alcohol, or separated according to Hoppe-Seyler, from one-thirtieth to one-fifteenth of the protein remains dissolved. All the proteins can be precipitated only by tannin in the cold or by copper hydroxide on heating. He further states that cow's milk contains about one-sixteenth of its nitrogen in non-protein form. Since our protein-free milk powder was equal to 50 per cent of the total solids of the milk, it should, if Munk's statements are correct, contain 0.48 per cent of non-protein nitrogen, thus leaving at the most only 0.28 per cent of the protein nitrogen, equal to 1.69 per cent of protein. Since 100 grams of the food mixture employed in our experiments contained 28.2 grams of protein-free milk powder, we can assume that at the most the food pastes thus made contained only 0.48 per cent of milk protein. The protein-free milk powder thus produced as above described left about 14.5 per cent of inorganic matter on ignition. This includes not only the inorganic constituents of the milk, although by no means in the combination in which they occur in the mammary secretion, but also the inorganic salts whch were formed by the addition of the hydrochloric acid used to precipitate the casein and also the sodium salts which resulted from neutralizing the milk serum with sodium hydroxide solution.

McCollum[56] doubted whether the significant nitrogen content of milk could be so easily removed as would appear, and was inclined to attribute the success attained with protein-free milk to the supplementary value of the nitrogen still present.

Mitchell and **Nelson**[57] endeavored to reduce the amount of residual nitrogen by the following procedure: Where the original method involves precipitation of the casein with slight excess of hydrochloric acid, filtration, heating the filtrate to boiling for one-half minute, filtering off the precipitate of lactalbumin, neutralizing the clear filtrate, and evaporating to dryness at a temperature not exceeding 70° C. upon neutralization of the filtrate after separation of the lactalbumin, a precipitate is always obtained, probably consisting largely of calcium phosphate. This was filtered off and the filtrate evaporated to dryness as usual. In this way preparations obtained contained on an average about 0.10 per cent less nitrogen than the preparations made after the original method of **Osborne** and **Mendel**. Presumably this reduction is largely if not entirely in the protein nitrogen.

These observers have found the preparation apparently as effective for periods of five or six months at least in covering the nutritive requirements of white mice for simple maintenance as rations containing the same amount of unfiltered production. They suggest as a better method of preparation of protein-free milk, precipitation

[56] **McCollum**, Am. J. Physiol. 25, 120; **McCollum** and **Davis**, J. Biol. Chem. 19, 250; 20, 647; **McCollum** and **Simmonds**, Am. J. Physiol. 46, 275, 1918.

[57] **Mitchell and Nelson**, J. Bi. Ch. 23, 459, 1915.

by trichloracetic acid. The optimum proportions were found to be 12 cc. of 50 per cent trichloracetic acid per 10 g. of milk powder, the filtrate being boiled for thirty minutes to an hour and filtered again. Two separate preparations were made according to this method, and found to contain 0.401 and 0.42% *N* respectively, equivalent to about one twenty-third of the total nitrogen of the milk. Tests failed to indicate with any certainty the presence of protein material. Although the tests have not been continued for a sufficiently long time to be entirely satisfactory, the product thus prepared seems to be as efficient for maintenance at least, as the preparations of **Osborne** and **Mendel.** In all cases **Mitchell** and **Nelson** used the dried centrifugalized milk put on the market by the Merrill-Soule Co., of Syracuse. This was dissolved in about twenty times its weight of distilled water, and treated accordingly to one or other of the above methods.

Kennedy[58] states that "protein-free milk" as prepared by the method of **Osborne** and **Mendel** contains either unprecipitated protein or peptides of considerable size as shown by amino nitrogen determinations before and after acid hydrolysis and the nitrogen distribution as well as by the increase in amino nitrogen after tryptic digestion.

Later, when it was recognized that the "protein-free milk" carried vitamins as well as inorganic salts, **Osborne** and **Mendel** evolved the following mixture of pure salts.[59]

	Gm.		Gm.
Calcium carbonate	134.8	Citric acid + H_2O	111.1
Magnesium carbonate	24.2	Ferric citrate + 1½ H_2O	6.34
Sodium carbonate	34.2	Potassium iodide	0.020
Potassium carbonate	141.3	Manganese sulphate	0.079
Phosphoric acid	103.2	Sodium fluoride	0.62
Hydrochloric acid	53.4	Potassium aluminum sulphate	0.0245
Sulphuric acid	9.2		

In the salt mixture used as reported in the Journal of Biological Chemistry **37**, 317, 1913, the proportion of sodium fluoride is given as 0.248. The other constituents are as given above.

The chemicals used were analyzed and allowance was made for moisture, etc. The acids were mixed and the carbonates and ferric citrate added to them. The traces of potassium iodide, manganese sulphate, sodium fluoride, and potassium aluminum sulphate were added as solutions of known concentration. The final resulting mixture was evaporated to dryness in a current of air at 90–100°C., and ground to a fine powder. This mixture with the addition of

[58] **Kennedy,** J. Am. Ch. Soc. **41,** 388, 1919.

[59] **Osborne and Mendel,** J. Biol. Chem. **15,** 317, 1913.

purified lactose was intended to reproduce as exactly as possible the composition of the "protein-free milk" with the exception of the traces of nitrogenous compounds and the vitamins.

McCollum and co-workers have experimented with a variety of salt mixtures, the following being noted: [60]

Salt Mixture I

	Gm.
Sodium chloride	0.173
Magnesium sulphate (anhydrous)	0.266
Sodium dihydrogen phosphate	0.347
Potassium monohydrogen phosphate	0.954
Calcium tetrahydrogen phosphate	0.540
Calcium lactate + 5 H_2O	1.300
Ferric lactate (Merck)	0.118

Salt Mixture II

	Gm.
Sodium chloride	0.146
Magnesium sulphate	0.225
Sodium dihydrogen phosphate	0.293
Potassium monohydrogen phosphate	0.805
Calcium tetrahydrogen phosphate	0.456
Ferric lactate	0.100

2.4 gm. of the above with 1.3 gm. of calcium lactate was used with each 100 gm. of ration.[61] Iodine was supplied in the drinking water once each week.[62]

Mixture I has been used satisfactorily by **Steenbock** [64] who states however that it is more complex than necessary, as he has secured excellent growth, reproduction, and the rearing of young on rations free from sulphates. **Mitchell** and **Nelson** [65] using mice as experimental animals were less successful. They say:

Our experiments with this salt mixture in experiments on mice have not been particularly encouraging. Rations containing all their mineral constituents in this artificial preparation have invariably produced unmistakable symptoms of malnutrition in the experimental animals, generally after two or three weeks time, though occasionally these symptoms have been deferred

[60] **McCollum** and **Davis**, J. Biol. Chem. 15, 167; 19, 250; **McCollum** and **Kennedy**, *Ibid.* 24, 494; **McCollum** and **Simmonds**, *Ibid.* 32, 355; **McCollum**, quoted by **McArthur** and **Luckett**, *Ibid.* 20, 163.

[61] **McCollum**, quoted by **McArthur** and **Luckett**, J. Biol. Chem. 20, 16B, 1915.

[63] **McCollum** and **Davis**, J. Biol. Chem. 20, 646, 1915.

[64] **Steenbock**, **Kent**, and **Gross**, J. Biol. Chem. 35, 67, 1918; **Steenbock**, **Boutwell**, and **Kent**, *Ibid.* 521; See also **Hogan**, *Ibid.* 27, 194, 1916.

[65] **Mitchell** and **Nelson**, J. Biol. Chem. 23, 459, 1915.

for a month or even two months. . . . It is possible that it rests simply on the fact that different species of animals have been used. Another possible explanation is that the discrepancy is due to the use by **McCollum** and **Davis** of wood shavings in their experimental cages and of paper excelsior by ourselves. It is possible that in the former case the wood shavings may have furnished some indispensable mineral constituents absent from the artificial salt mixture which constituted a part of the rations. We cannot agree with the statement of **McCollum** and **Davis** [66] that they do not look upon the consumption of a small amount of wood fibre as objectionable to any greater degree in this type of experiment than is the feeding of agar agar. Also the availability and nutritive value of the nitrogenous and mineral substances of the wood cannot be as lightly disregarded, we believe, as they have been by these investigators.[67]

A study of the inorganic elements in nutrition was carried out by **Osborne** and **Mendel** by preparing a large variety of salt mixtures in which one or more of the elements was omitted and replaced by increments of the remaining ones so as to maintain the balance of the acids and bases as nearly as possible. This was fed to the rats on an otherwise salt-free diet, and the effect on their growth noted. Good growth was possible on foods containing only traces of magnesium, sodium and chlorine, less than 0.04 per cent of either sodium or chlorine in the food being sufficient to enable rats to complete their growth. When both sodium and potassium were lacking growth stopped, but when only one of these elements was missing growth was nearly or quite normal. Restriction of either calcium or phosphorus causes prompt restriction of stoppage of growth. Magnesium could not replace calcium even when given in large quantities. **Osborne** and **Mendel**, commenting on the fact that the growing animal can fully supply from inorganic sources its requirements for various of the elements emphasizes anew that it is unnecessary to consider the presence of calcium, phosphorus, and iron, for example, in natural food to the degree that is currently believed. Any shortage of an essential inorganic element can be suitably remedied under ordinary conditions by the use of its salts. For feeding farm animals, where the lack of calcium and prosphorus in their grain rations is always encountered, the demonstration that complete nutrition can be attained upon diets in which the inorganic ingredients are supplied in the form of their commercial salts has a significance that is just beginning to be appreciated.

Some interesting observations have been made by **Sherman** and **Pappenheimer** (J. Exptl. Med. 34, 189, 1921) respecting the effect of phosphates and lime salts on rickets. A diet of 95% patent flour,

[66] **McCollum and Davis, J. Biol. Chem. 19, 250, 1914.**

[67] **Osborne and Mendel, J. Biol. Chem. 34, 131.**

3% calcium lactate, 2% common salt and also a diet having the same proportion of flour, 2.9% calcium lactate, 2% salt and 0.1% iron citrate regularly induced rickets in young rats. The substitution of 0.4% potassium phosphate for a small portion of the calcium lactate in the diet very curiously prevented the development of rickets. An examination of the content of calcium in the bodies of the rats showed those receiving the potassium phosphate had assimilated a greater proportion of calcium than those which developed rickets. The results obtained perhaps may not be due to the greater assimilation and deposition of calcium. There may be a quantitative relationship between the lime and phosphorus rather than an actual deficiency of either of them.

A pathological condition bearing fundamental resemblances to rickets of the human being resulting from diets low in phosphorus and fat-soluble *A* has been observed by **Shipley, Park, McCollum** and **Simmonds** (Bull. Johns Hopkins Hosp. 32, 160, 1921). Two diets, low in fat-soluble *A* and phosphorus produced in the majority of the young rats placed upon them pathological conditions of the skeleton having a fundamental resemblance to rickets. The changes are not identical, however, with that disease as it usually manifests itself in the human being. The chief difference consisted in the presence of scattered or irregular deposits of calcium salts in the cartilage and metaphysis. When the deficiency in phosphorus is compensated by the addition of a complete salt mixture containing PO_4 ion, the deficiency in fat-soluble *A* still existing, no pathological changes of a rachitic nature developed. The addition of the PO_4 ion to the diets deficient in it and in the factor *A* prevented, therefore, the development of any changes of a rachitic-like nature. Thus the PO_4 ion in the diet may be the determining influence for or against the development of rickets. If the PO_4 ion content of the diet is sufficiently high, a deficiency of *A* cannot cause these changes in the skeleton. A deficiency in *A* is held not to be the sole cause of rickets. Conversely, it is necessary that the diet be low in its content of phosphorus, all other factors, except *A*, being optimal, for these conditions to develop. Since the addition of the PO_4 ion to the diet prevented the development of these changes in the skeleton, but had no effect in preventing xerophthalmia, it seems permissible to infer that xerophthalmia and rickets do not have an identical etiology. The above results do not exclude *A* from consideration as an etiological factor in the production of rickets and kindred diseases, since the level of the blood PO_4 ion is, in all probability, determined in part by the amount of *A* available.

CHAPTER IV

THE DISTRIBUTION OF THE VITAMINS

MUCH information of a general character as to the distribution of the vitamins has been gathered during the last decade, but our knowledge is still far from satisfactory. Only very recently has there been any attempt to make the tests quantitative and lack of standardized methods has made it impossible to compare with any degree of accuracy the results obtained by different experimenters. **Steenbock** and **Gross**[1a] consider that the time is not yet ripe to warrant a general classification of foods into groups (1) rich in, (2) poor in, and (3) free from fat-soluble vitamin; as general methods of experimentation, especially in reference to vitamin content of the basal food ingredients,[2] period of observation, and control of experimental animals, differ so greatly in different laboratories that the conclusions of the different observers are hardly comparable. Furthermore, there is much reason to believe that the variation in the fat-soluble vitamin content of naturally occurring food materials, even when harvested at the same stage of development, is tremendous. No doubt, according to Steenbock and Gross, there lies here the foundation for many interesting correlations in the functional role of specific substances in both plant and animal kingdoms, the significance of which can scarcely be predicted.

The results of tests of the different foodstuffs so far as they have been obtained are collated in tables appearing in the appendix, with brief comments made by the observers, but, owing to the difficulties referred to above, no attempt has been made to compare the relative amounts present. The comparatively few researches in which more precise methods have been employed are discussed in greater detail in the text.

Fat-soluble *A*, as suggested by **McCollum's** nomenclature, is commonly associated with natural fats. The fact that it was first detected in butter-fat, and that this is a particularly convenient source, has led to what is perhaps over-emphasis upon the value of this food-

[1] For tables, see Appendix.

[1a] **Steenbock and Gross**, J. Biol. Chem. 40, 503, 1919.

[2] See also **Drummond and Coward**, Bioch. J. 14, 661, 1920.

stuff. While butter is commonly eaten in greater quantity than any of the other foods in which *A* is found, it is by no means essential as a source of this factor, since as will be seen later, certain vegetable products equal or surpass butter-fat in this respect, and on the other hand, **Steenbock, Boutwell** and **Kent**[3] have called attention to the wide variation possible in different butters and suggested a possible explanation.[4]

"In the course of many experiments designed to demonstrate the vitamin content of butters we have been impressed with variations in the amount of vitamin present. These variations are not the result of heat treatment as in some cases the exposure to heat in the preparation of the fat was reduced to the minimum necessary to secure a clear sample. We surmised that possibly the rations on which the butter-fat had been produced might be responsible. On these general premises lard could be said to owe its deficiencies in vitamin to the poverty of the grains in this dietary essential. Similarly beef fat would owe its correspondingly greater vitamin content to the greater vitamin content of the roughages such as clover and alfalfa so extensively fed for beef and milk production. As yet experiments in this direction have not progressed sufficiently to warrant this generalization. One fact, however, is clear. Vitamin content of the ration on which butter-fat is produced is not the only factor to be considered. One sample of butter-fat obtained from a cow fed exclusively on alfalfa hay was found to contain no demonstrable amounts of vitamin. This butter had been kept in an unsalted condition in a poorly iced refrigerator for about three weeks. In this time some molds had developed on its surface which necessitated discarding part of the material. . . . Questions of influence of feed, storage conditions, and temperatures used in the renovation of inferior products and even the method of use of the product in the home must be taken into consideration when depending upon butter-fat as a sole source of fat vitamin in the dietary."

It is an interesting fact that butters rich in pigments usually contain more vitamins than those less highly colored.[5] According to **Stephanson,**[6] however, extraction of the coloring matter of butter by treatment with wood charcoal does not affect the vitamin content.

Beef fat contains some *A*, the more liquid portion (oleo oil) having the highest concentration. This accounts for the presence of vitamins in oleomargarines made from animal fats, some of which have been reported by **Halliburton** and **Drummond**[7] to be equivalent to butter fat in this respect. Oleomargarines from vegetable fats on the other hand have been found to be poor, if not entirely lacking in *A*.

[3] **Steenbock, Boutwell** and **Kent,** J. Biol. Chem. 35, 519, 1918.

[4] See also **Dutcher, Kennedy** and **Eckles,** Sci. 52, 588, 1920, and **Drummond** and **Coward,** Bioch. J. 14, 668, 1920, on the influence of the diet of cows upon the fat-soluble and water-soluble vitamins in cow's milk.

[5] **Steenbock, Sell** and **Buell,** J. Biol. Chem. 47, 89, 1921.

[6] **Stephenson,** Bioch. J. 14, 715, 1920.

[7] **Halliburton** and **Drummond,** J. Physiol. 51, 235, 1917.

The fat present in egg-yolks is rich in *A*, as is also cod-liver oil, and it has been suggested that the long-recognized restorative value of milk, eggs and cod-liver oil may depend upon their vitamin content.

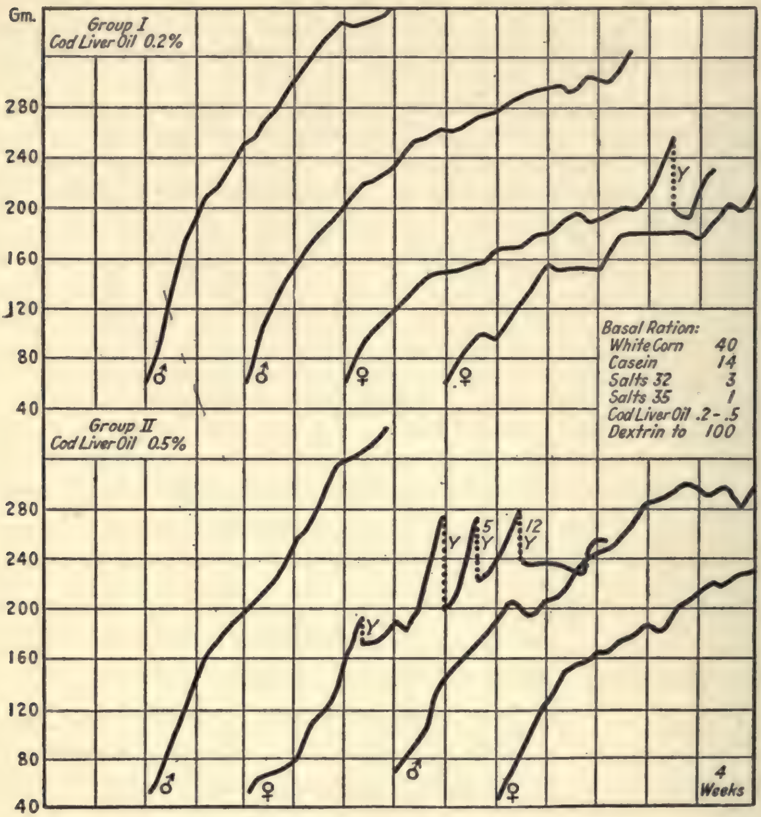

FIG. 3. — The remarkable growth-promoting property of small amounts of cod liver oil as a source of fat-soluble vitamin is illustrated by the above growth curves. White Indian corn was used as a source of water-soluble vitamin as it has been shown in numerous experiments to lead to nutritive failure as a source of fat-soluble *A* and yet it furnishes plenty of the water-soluble vitamin for normal growth.

Courtesy of Prof. H. Steenbock, M. T. Sell, M. V. Buell and Journal of Biological Chemistry (47, 102, 1921).

A has been found in all crude fish oils, which have been tested, but cod liver and whale oil appear to be the richest.[8]

[8] **Zilva** and **Miura** (Lancet. 1921, I, 323) used a quantitative method of estimation of the fat-soluble accessory factor in cod-liver oil and butter. It

Lard has been generally believed to be entirely lacking in *A* and for this reason has been regularly used as a component of the basal ration in testing for the presence of *A* in other foodstuffs, but **Daniels** and **Loughlin**[8a] have reported that it actually contains a certain amount, although this is only demonstrable when the lard is fed at a high level (28 per cent). On a diet of 18 per cent of casein (extracted for 48 hours with ether in a Soxhlet), 28 per cent of lard, 7 per cent of salt mixture, 47 per cent of corn starch, and the water-alcoholic extract of 9 gm. of wheat embryo (previously extracted with ether) they secured good growth and reproduction of rats over a period of 5 months, but when the lard was reduced to 21 per cent growth ceased after about two months. If this is correct, then *A* is present to some extent at least in all fats of animal origin so far tested but as yet these results lack complete confirmation.

A series of careful investigations has been carried on by **Drummond**[9] to determine the nutritive value of lard and lard substitutes from the standpoint of vitamins. This work was undertaken in view of the fact that no entirely satisfactory explanation of the deficiency of lard in vitamin content had so far been advanced by any investigator.

In this connection it is appropriate to note the results of some experiments conducted by **Drummond** on the digestibility of these products. All edible oils and fats with rare exceptions are well digested and absorbed by the mammalian alimentary tract. The following table will show the melting point and digestibility of lard and some of its substitutes:

Sample	Approx. M.P. °C.	Length of trial in days	Average coefficient of digestibility — Per cent
Neutral lard I	37.50	10	97.70
Neutral lard II	37	10	98.20
Hardened cotton-seed oil, lard substitute I	40	7	96.10
Hardened oil, lard substitute II	38	10	97.20
Lard substitute A, compound lard	36	7	96.40
Lard substitute B, compound lard	37	7	96.70
Lard substitute C	37	7	97.50

was found that the former was much more potent. The high potency is contained particularly in the crude preparations of cod-liver oil.

Steenbock, Sell and **Buell** call attention to the high content of fat-soluble *A* in cod liver oil although this oil contains very little yellow pigment. (J. Biol. Chem. 47, 89, 1921).

[8a] **Daniels** and **Loughlin**, J. Biol. Chem. 42, 359, 1920.

[9] **Drummond, J. S. C. I. 1921, 81 T.**

From our present knowledge of the vitamin-content of fats and oils we recognize that the results tabulated above are likely to lead to erroneous conclusions. Judging these fats from the standpoint of their digestibility would place them about on parity. But rating foods on calories and digestion coefficients without considering the vitamin content is no longer sound. Recent researches have demonstrated that the amount of vitamin *A* present in the body fat of an animal is determined largely by the diet which the animal has received. Apparently the animal organism does not possess the power to synthesize vitamins, but can store up a reserve in certain parts of the body when a surplus is provided in the diet.

In order to throw light on these points, experiments were made with young pigs reared on dietaries some of which were practically devoid of vitamin *A* while others were supplied with an ample amount of that factor.[10]

Certain animals from each lot, after several months on the special diets, were slaughtered and samples of leaf fat (peri-nephritic fat) and back fat were removed. By means of the biological method these products were tested for the presence of vitamin *A* with the following interesting results. The body fats of swine fed on dietaries furnishing ample supplies of vitamin *A* contained that substance, but its presence could not be demonstrated in the fats derived from animals fed on the foods deficient in vitamin *A*.

Drummond[11] examined the various processes of lard manufacture in order to ascertain their effect on the vitamin *A* originally present. The destruction of *A* at high temperatures was at one time believed to be due to the heating alone,[12] but more recently it has been shown that temperatures up to 120° C. do not inactivate the vitamin unless there is contact with air or oxygen. It would, therefore, appear that the destruction is probably due to changes of an oxidative nature.[13]

The older methods of lard manufacture, which are still used to some extent, are essentially modifications of the original farmhouse process in which the fat is rendered at low temperature, about 70° C., and separated in a simple manner from the connective tissue and water, etc. The more up-to-date processes involve a certain amount of aeration at higher temperatures. Stirring is employed during melting and after the separation of the layers, the fat is stirred at

[10] **Drummond, Golding, Zilva** and **Coward**, Biochem. J. 1920, 14, 742.

[11] J. S. C. I. 1921, 81T.

[12] **Steenbock, Boutwell** and **Kent**, J. Biol. Chem., 1918, 35, 577; **Drummond**, Biochem. J. 1919, 13, 81.

[13] **Hopkins**, Biochem. J. 1920, 14, 725; **Drummond**, Biochem. J. 1920, 14, 734; **Zilva**, Biochem. J., 1920, 14, 740.

the temperature of 102° C. for ten to fifteen minutes to remove moisture.

By actual experiment it was found that the lard prepared from hog-fat rich in vitamin *A* by the *simple* rendering process contained practically all the vitamin originally present in the fat. However, an active sample of hog-fat was found to have lost practically all of its vitamin *A* after being converted to lard by the rendering process more commonly used, employing agitation and higher temperatures.

Oleo-stearin also appears to be generally deficient in vitamin *A*. Beef fat normally contains appreciable quantities of this indispensable dietary constituent,[14] and these observers found on crystallization of beef fat from alcohol that the vitamin is concentrated in the mother liquors together with the glycerides of lower melting point. This explains why in the process of separating oleo-stearin from premier jus the greater part of the vitamin passes through with the expressed oleo oil. The usual process of oleo oil preparation would hardly be likely to affect the amount of vitamin present.

Considerable variations in the vitamin content of oleo oil may be encountered, but these are in all probability due to the seasonal variations in the diets of the animals. Oleo-stearin is practically always of low vitamin value. The nutritive value of compound lard will therefore be much influenced by the vitamin content of the constituents employed in its compounding.[15]

Lean meat and fish are probably entirely deficient in *A*.[15a]

Vegetable fats and oils seem in general to be poor in *A*, although as **Drummond** points out,[16] no hard and fast line can be drawn between these two classes of oils and fats when their value as sources of the fat-soluble vitamin *A* is considered. Certain vegetable oils, as for example palm and yellow maize oils, are in the crude condition good sources of the vitamin. Both animal and vegetable oils and fats tend to lose vitamin *A* on refining. **Bolton** observes that it would be desirable to find a method whereby the manufacturer could first separate the vitamins from oils, then carry on the refining process, and re-introduce the vitamins which otherwise would be destroyed by the process.[17]

[14] **Osborne and Mendel,** J. Biol. Chem., 1915, 20, 379.

[15] **Drummond,** J. S. C. I. 1921, 81T.

[15a] In rats, dead as a result of vitamin-free diets, both fats and lipoids may be completely lacking in the "lipoid gland tissue." It is suggested by **Cramer** (Proc. Physiol. Soc.; J. Physiol. 54, ii, 1920) that this lipoid gland tissue forms an important deposit of one or more of the vitamins.

[16] **Drummond and Coward,** Bioch. J. 14, 668, 1920.

[17] J. S. C. I. 1921, 81 T.

Comparing the different plant structures, the leaves are undoubtedly richest and the seeds as a general rule poorest in *A*, although flax and millet seed appear to be an exception.

Of the leaves which are used for human food, spinach seems to be the most efficient. **Steenbock** and **Gross**[18] found that five per cent of lettuce, spinach, and chard in the basal ration furnished enough *A* for long continued though somewhat subnormal growth. Few young were produced and none were reared but in no case was there any appearance of the xerophathalmia commonly associated with deficiency of *A*. Judging by the appearance of the animals, spinach seemed to be the most satisfactory and lettuce the poorest, of this group. **Osborne** and **Mendel**[19] also noted that spinach leaves appeared to be richer in *A* than most of the products used in an ordinary diet.

The grasses clover, alfalfa, and timothy have all been found exceptionally rich in *A*, especially when cut before reaching full maturity and carefully cured. **Steenbock** and **Gross**[20] found it possible to secure growth at the normal rate on a ration carrying five per cent of alfalfa as the only source of *A*. Young were successfully reared, although the time for rearing was prolonged eight to ten days beyond the normal, thus giving indisputable evidence of the richness of alfalfa in the fat-soluble vitamin. How much less than five per cent of the ration might have been constituted of alfalfa and still have produced results such as the above was not determined, but Steenbock and Gross were inclined to think from the curves of growth and the behavior of the young that they had reached approximately the lowest level possible with their material. With five per cent of clover as the source of *A*, growth approximating normal was secured, and young were reared though at a subnormal rate of growth.

Osborne and **Mendel** tested the value of various plant and animal products[21] by determining the amount necessary to promote renewed growth in rats which had declined on a diet deficient in *A* but suitable in all other respects. Their basal ration was composed of meat residue, 19.6 per cent, salt mixture of 4.0 per cent, starch 52 per cent, lard 24 per cent, and yeast 0.4 gm. daily. On such a diet, rats soon fail to grow and then begin to decline rapidly in body weight, but unless the damage has been allowed to go too far, restoration to health and renewed growth can be brought about by replacing part of the lard

[18] **Steenbock** and **Gross**, J. Biol. Chem. 41, 149, 1920.
[19] **Osborne** and **Mendel**, J. Biol. Chem. 37, 188, 1919.
[20] **Steenbock** and **Gross**, J. Biol. Chem. 41, 149, 1920.
[21] **Osborne** and **Mendel**, J. Biol. Chem. 41, 551, 1920.

by butter-fat or some other potent source of *A*. When the other dietary factors were satisfactory they found 0.5 gm. of butter-fat (five per cent of the ration) sufficient for this purpose, but made no tests to determine whether this amount represents the minimum. The superior efficiency of some plant products as a carrier of the *A* vitamin is indicated by the fact that as little as from 25–70 mg. per day of ether extract of clover, timothy, alfalfa or spinach induced recovery and rapid growth in animals which were suffering from lack of this vitamin. A similar amount of dried cabbage was not sufficient, although there was evidence of the presence of some *A*. **Steenbock** and **Gross** [22] were not very successful in attempting to use fresh cabbage as a source of *A*, owing to the fact that cabbage is liable to cause digestive disturbances which result in a decreased food intake and consequent complications in the relation between growth and diet. Fifteen per cent of cabbage in the ration was sufficient to prevent decline and even to promote growth, though at a subnormal rate, but the fact that addition of butter-fat produced prompt acceleration in the rate of growth indicated that this amount of cabbage did not furnish the optimum amount of A.

Tubers and roots differ considerably, but may be regarded for the most part as intermediate between leaf and seed. White potatoes are not rich in the factor, being about equal to the cereal grains in this respect, according to **McCollum, Simmonds** and **Parsons**,[23] while the sweet potato has a much higher concentration of *A*, 15 per cent in the diet being sufficient to permit of normal growth and reproduction. Carrots are about equivalent to sweet potatoes as a source of *A*, but the dasheen, which is a vegetable of very similar type, is apparently almost devoid of it,[24] and the sugar beet and mangel contain very little.[25]

Most seeds contain some vitamin *A*, but very few are rich in this factor.[25a] Flax and millet seeds and the Georgia velvet bean have an exceptionally high content of *A*, but the ordinary edible seeds contain

[22] **Steenbock** and **Gross**, J. Biol. Chem. 41, 149, 1920.

[23] **McCollum, Simmonds** and **Parsons**, J. Biol. Chem. 36, 197, 1918.

[24] **Steenbock** and **Gross**, J. Biol. Chem. 40, 501, 1919.

[25] See also **Steenbock** and **Gross**, *Ibid.* 40, 529, 1919, and **Osborne** and **Mendel**, *Ibid.* 41, 555, 1920.

[25a] White rats fed rations in which all the protein (18.4%) was supplied by tomato-seed press cake grew at the normal rate. Water-soluble *B* and fat-soluble *A* vitamins apparently were furnished in adequate amounts for normal growth when one-half the ration consisted of tomato-seed cake, the other half being made up of purified products. **Finks** and **Johns**, Am. J. Physiol. 56, 404, 1921.

relatively little.[26] **Coward and Drummond**[27] found that nuts such as the Brazil nut, peanut, walnut, and almond, furnish only an insignificant amount of *A* despite their high fat content.

Fruits have not been very extensively investigated as to their content of *A*, but bananas contain at least some of this vitamin, while lemons, grape fruit and oranges are apparently completely lacking. Tomatoes are unusually rich in *A*, dried tomato being more efficient, weight for weight, than butter-fat.

Various factors influence the value of milk and butter as sources of vitamin *A*. **Drummond, Coward and Watson**[28] have studied these variations. The diet of the cow is undoubtedly the chief cause of variations in the amount of vitamin *A* in milk. Colostrum is richer than milk; butter is somewhat poorer, partly owing to mechanical loss and partly to destruction. "Blowing" and other methods of butter-making which involve exposure to air at high temperatures, may cause a loss of vitamin *A*. The vitamin content of butter produced in winter is low, sometimes very low, because the cattle are stall-fed on dry feeds of hay, roots and cake; even a summer drought may lower the vitamin content. Storage of butter does not lower the vitamin *A* content unless there is oxidation, nor does development of rancidity *per se*. "Renovation" of rancid butter will entail further loss of vitamin, if the methods employed cause oxidation.

Stammers reports that bran contains vitamin *B* and also some vitamin *A*.

McCollum and others have endeavored to correlate the distribution of vitamins with the function of the plant organ.[30] It has been found that the leaf of the plant is several times richer in fat-soluble *A* than are the wheat, oat, and corn kernels. Certain seeds approximate the value of the leaf in this substance. Hemp seed is distinctly better than those just named, but flaxseed and millet seed are still richer than hemp seed and may readily be incorporated in the diet

[26] A series of careful feeding tests conducted by **Sure** and **Read** has established the fact that the Georgia velvet bean seed of early speckled variety is abundant in fat-soluble *A* but low in water-soluble *B*. This seed when fed raw is injurious to the growth of young rats. However, the injurious effect can be destroyed to a great extent by autoclaving the seed for one hour at 15 pounds pressure without destroying the vitamins. (J. Agricultural Research, 22, No. 1, 1921). According to **Finks** and **Johns** (Am. J. Physiol. 57, 61, 1921) the nutritive value of the proteins from Chinese and Georgia velvet beans is not satisfactory.

[27] **Coward and Drummond**, Bioch. J. 14, 665, 1920.

[28] **Drummond, Coward and Watson**, Biochem. J., 1921, 15, 540, 1921.

[29] **Stammers**, Biochem. J. 15, 489, 1921.

[30] **McCollum, Simmonds and Pitz**, J. Biol. Chem. 30, 18, 1917.

in amount sufficient to meet the needs of an animal for the fat-soluble *A* during growth.

It is interesting to note that the content of the fat-soluble *A* is highest in those seeds which are smallest (flax and millet). Possibly this may be in some measure related to the relatively large proportion of germ as compared with endosperm in such seeds. The endosperm is in great part to be likened to a mixture of purified proteins, carbohydrates, and fats, while the germ is relatively rich in functioning plant cells as well as fats, carbohydrates, etc. The differences in the amount of stored food material contained in the germ in various seeds render it impossible to make such a comparison very accurate. It has been suggested that the differences in the dietary properties of the entire seed as contrasted with the embryo, and of polished rice as contrasted with hulled rice, may lie in part in the association of *A* and *B* with the functioning cell. In like manner, it is possible to account for the exceptional richness of the leaf in these two dietary principles. The leaf is the seat of great synthetic activity and comprises a relatively large amount of functioning cells associated with those substances which may be considered as the equivalent of purified protein, carbohydrate, and fats.

This point of view is criticized by **Steenbock** and **Gross**[31] who point to the great variation in vitamin content of organs so closely associated in function as tubers and roots. These investigators have adopted a hypothesis of their own, according to which *A* is associated with the occurrence of certain yellow plant pigments. In support of this hypothesis it is pointed out[32] that experiments with eight varieties of corn have indicated that white corn contains practically no fat-soluble vitamin while yellow corn contains a sufficient amount to permit normal growth and reproduction in the rat. Colored roots such as carrots and sweet potatoes have been found to contain much fat-soluble vitamin, while the uncolored sugar beets, mangels, dasheens and Irish potatoes show little of it. Experiments with commercial oleo oils show that those most highly pigmented are also richest in fat-soluble vitamin, while the least pigmented are poorest in this growth essential. The fact that the yellow pigment in butterfat disappears simultaneously with the destruction of the vitamin by heating may or may not be significant, but is at least a suggestive

[31] **Steenbock and Gross**, J. Biol. Chem. 40, 503; 41, 149. See also **Steenbock and Boutwell**, J. Biol. Chem. 41, 81, 163; 42, 131. **Steenbock, Sell, Nelson, and Buell**, Proc. Soc. Biol. Chem. J. Biol. Chem. 46, xxxii, 1921. **Steenbock, Sell, and Buell**, J. Biol. Chem. 47, 89, 1921.

[32] **Steenbock**, Science, 50, 352, 1919.

coincidence.[32a] The occurrence of certain materials which contain as much fat-soluble vitamin as yellow corn, but which are much less pigmented might be due to the presence of a leuco compound of the yellow pigment.[33]

Ripe peas of green color were found richer in vitamin *A* than those of a yellow color. The former also contained more yellow pigment than the latter.[34]

Rosenstein and **Drummond**[35] also reported that the *A* vitamin was to be found contained in the same foodstuffs that contained lipochromes, and concluded that it was probably associated in some manner with pigments of the carotin type. Further study, however, showed that the presence of yellow lipochromes was unreliable as an indication of the presence of *A* and **Drummond** has abandoned this hypothesis.[36]

Palmer[37] disagrees with **Steenbock's** assumption and points to the case of cottonseed oil which is golden yellow in color and rich in carotinoids, but is remarkably free from vitamin. The same seems to be true of corn oil. He also adduces the fact that he was able to raise a flock of chickens to maturity on a carotinoid-free diet. These chickens laid carotinoid-free eggs, which hatched into a second generation of chicks, free from carotinoid. He states that either the fat-soluble vitamin and the yellow plant pigments are not related physiologically or the fat-soluble requirement of fowls differs from that of animals.

It may be noted, however, that **Palmer's** basal ration contained abundance of pork liver, which was believed to be free from carotinoids but fairly rich in *A*, whereas **Rosenheim** and **Drummond**

[32a] In beef-fat and butter-fat, according to **Steenbock, Sell** and **Buell,** the content of *A* does not closely parallel the pigment content. However, both vitamin and pigment are closely associated in these fats and hence the most highly pigmented fats are generally richest in vitamin *A*. (Steenbock, Sell and Buell, J. Biol. Chem. **47,** 89, 1921).

[33] **Willaman** (Am. Food Jour., Oct. 1921) emphasizes the virtues of yellow vegetables based on the observations that yellow color and vitamin *A* practically always occur in parallel amounts. The list includes sweet potato, Irish potato, mangel, squash, dasheen, sugar beet, many varieties of corn and peas, spinach, chard, lettuce, cabbage, alfalfa, clover, timothy and tomato fruits.

[34] **Steenbock, Sell** and **Boutwell,** J. Biol. Chem. **47,** 303, 1921; J. S. C. I. 1921, 713A.

[35] **Rosenstein** and **Drummond,** Lancet 1920, 1, 862. See also **Drummond,** Bioch. J. **13,** 81, 1919.

[36] **Drummond** and **Coward,** Bioch. J. 1920, 14, 668.

[37] **Palmer,** Sci. 50, 501, 1919. **Palmer** and **Kempster,** J. Biol. Chem. **39,** 299, 1919; **Palmer** and **Kennedy,** *Ib.* **46,** 559.

(l. c.) state that liver tissue was found to contain, besides carotin and xanthophyll, a substance which gives certain reactions similar to those given by the lipochromes, but not identical with any known member of that class.

From later experiments **Palmer** and **Kennedy**[38] report that rats will grow and reproduce on ewe milk fat containing only 0.00014 per cent of carotin, or even with carotinoid-free egg yolk as the sole source of vitamin *A*.

Vitamin *B* appears to be present to some extent in almost every natural foodstuff. Yeast was considered by **Funk** to be the most efficient source, but the Lister Institute workers[39] place pressed yeast fourth on the list of substances effective for prevention of beriberi; rice and wheat germ and lentils, being apparently quantitatively superior to it.[40] According to **Seidell**[41] fresh pressed yeast is inferior to autolyzed yeast for this purpose.

Bread made from mixtures of wheat flour and soya bean flour was found by **Johns** and **Finks**[41a] to have higher nutritive qualities than that made from wheat flour alone. Feeding tests on rats were made with 15 to 25 per cent soya bean flour in the wheat flour and these trials showed that proteins and vitamin *B* in amounts sufficient for normal growth were furnished by the ration. In fact the mixed proteins proved two or three times as effective for growth as wheat proteins alone.

Seeds in general contain an abundance of *B*. As small an amount as 15 per cent of whole wheat as a source of *B* is sufficient for complete growth in the rat, and so promotes well being as to induce production of nearly the normal number of young, but this amount is not great enough to enable the young to reach weaning age without pronounced nervous disturbances ending in death.[42] Beans do not differ greatly from the cereal grains, and dried peas are also markedly efficient.

Dried seeds have varying, generally small, quantities of vitamin *A*, which are not increased on germination. Green leaves form large quantities from inorganic salts, but the vitamin is not synthesized in the absence of chlorophyll. The vitamin is synthesized by green algae and, to a lesser extent, by red algae, but not by mushrooms.

[38] **Palmer and Kennedy,** l. c.

[39] **Funk,** Bioch. Bull. 5, 1, 1916.

[40] Med. Res. Com. Report No. 38.

[41] **Seidell,** J. Biol. Chem. 29, 145, 1917.

[41a] **Johns** and **Finks,** Am. J. Physiol. 55, 455, 1921.

[42] **McCollum, Simmonds and Pitz,** J. Biol. Chem. 28, 211, 1916–17; See also **Osborne** and **Mendel,** *Ibid.* 37, 590, 1919.

Vitamin *A* of green leaves is not associated with proteins; it may be extracted in the fat removed by solvents and appears in that fraction of the fat which resists saponification.[43]

With regard to the distribution of the vitamin within the seed, **Chick** and **Hume** state that in cereals the anti-beriberi vitamin is mainly deposited in the germ or embryo of the grain and to a less extent in the bran. White wheaten flour and polished rice, which consist of the endosperm of the grain, are deficient in this vitamin. Wheat endosperm, after removal of the aleurone layer in the ordinary milling process, constitutes white flour, which is deficient in antineuritic vitamin and will produce polyneuritis in pigeons or beriberi in man, if used as an exclusive diet, acting in a manner identical with the behavior of polished rice. In both the rice and wheat grain, this vitamin is contained in the germ or embryo, being present to a lesser degree in the bran (pericarp and aleurone layer), probably in the aleurone layer. The embryo of corn grain possesses marked antineuritic properties; both the scutellum and the plantlet contain the antineuritic vitamin.[44] Rice germ, according to these investigators, is about twice as efficient as wheat germ, weight for weight, as a preventive of beriberi.

This theory as to the distribution of the anti-beriberi factor in seeds is confirmed by other experimenters,[45] and **McCollum**[46] asserts in general that the cell-rich tissues of the plant such as the germ are better sources of *B* than are the entire seeds, but these in turn are better than is the endosperm.

All these investigators made use of the commercial embryo of the grain for their tests. **Osborne** and **Mendel**[47] have shown that whereas commercial wheat embryo is rich in *B*, if the embryo be very carefully separated by hand from the remainder of the seed it is a much less efficient source of this factor than has been supposed. On diets including as much as 150 mg. of pure wheat embryo per day as the only source of *B* they secured perfect maintenance of rats but no growth. On the other hand the residues from which the embryo had been carefully removed proved quite as effective in promoting growth as did the whole wheat kernel. When the embryo-free grain was divided into sections and fed separately it appeared that the end near the embryo is more efficient than the remainder of

[43] **Coward** and **Drummond**, Biochem. J., 15, 530, 1921.

[44] **Chick** and **Hume**, J. Roy. Med. Corps, 29, 121, 1917.

[45] **Voegtlin, Lake** and **Myers**, U. S. Pub. Health Reports 33, 647, 1918; Am. J. Physiol. 48, 504, 1919.

[46] **McCollum, Simmonds** and **Parsons**, J. Biol. Chem. 37, 287, 1920.

[47] **Osborne** and **Mendel**, J. Biol. Chem. 37, 557, 1918.

the seed, although the latter is by no means devoid of activity. They conclude that the vitamin is located in the endosperm, but not uniformly distributed through it, the high vitamin activity of the commercial embryo being probably due to the adherence of a considerable proportion of the softer parts of the endosperm which surround the embryo proper. According to Osborne and Mendel, this conclusion is not at variance with their experiments with wheat flour or commercial embryo meal, nor with **Voegtlin, Lake** and **Myers'** discovery that patent flour is practically free from vitamin. In making flour by gradual reduction the grain is broken into successively smaller particles, a bolting process being interposed between the breaks. The finer particles, which represent the more friable part of the endosperm, are thus sifted out and the larger and harder particles which remain on the sieves are ground into patent flour. If the vitamin is in fact concentrated in the softer parts of the endosperm the patent flour may be expected to be nearly free therefrom and the lower grades to be correspondingly richer in this food factor.

An attempt has been made to correlate the presence of vitamins with the distribution of phosphorus in cereals. **Fraser** and **Stanton**[48] concluded that a diet consisting mainly of rice with a phosphorus pentoxide content of less than 0.4 per cent would not furnish sufficient antineuritic body for safety. **Voegtlin** and **Myers** assert that:

" The phosphoric anhydride determination of wheat and corn products yields fairly satisfactory information as to the content of these products in accessory foods. A low phosphoric anhydride content indicates that the product is poor in vitamins.[49]

Voegtlin, Sullivan and **Myers**[50] proposed a minimum phosphorus pentoxide content of 0.5 per cent as a standard for corn products, and of 1 per cent for wheat flour. **Green** objects to this standard.[51] He admits that the examination of maize-milling products by dietetic experiments using the pigeon as discriminant, indicates that the distribution of vitamin in the corn kernel follows the distribution of phosphorus pentoxide whenever any given sample of grain is taken into consideration, but claims that the parallelism does not hold between different samples of grain, and that no difference in vitamin content could be detected by pigeon analysis in a series of samples

[48] **Fraser** and **Stanton,** Studies from the Institute of Medical Research, Federated Malay States, 1909. The etiology of beri-beri.

[49] **Voegtlin, Lake** and **Myers,** U. S. Public Health Repts., 33, 647, 1918; **Voegtlin** and **Myers,** *Ibid.* 33, 911, 1918.

[50] **Voegtlin, Sullivan** and **Myers,** *Ibid.* 31, 935, 1916.

[51] **Green,** S. Af. J. Sci. 14, 519, 1918, Chem. Abs. 12, 2600, 1918.

of whole corn varying in phosphorus pentoxide content from 0.35 to 0.71 per cent. In these samples the "indicator limit" of phosphorus pentoxide for milled meals on the border-line of efficiency, would vary from 0.23 to 0.46 per cent. From this it would seem impossible to use phosphorus pentoxide in milled products as indicator of vitamin efficiency unless the phosphorus pentoxide content of the original mother-grain is known. This information is rarely available, and the determination of phosphorus pentoxide as a general analytical guide to efficiency, as advocated by **Voegtlin, Sullivan** and **Myers** would be impractical according to **Green.** Their standard of 0.5 per cent for phosphorus pentoxide corn meal would condemn more samples than it passed, including the majority of perfectly efficient South African meals. Simple microscopic examination of a meal, to gage the extent of milling, would be a safer guide than the phosphorus pentoxide standard.

By taking "average pigeon requirements" as standard for comparison, and stating this as 100, **Green** has assigned "vitamin indices" to certain cereal products. On this basis whole corn (maize) works out at about 160 to 180—i. e., contains over 60 per cent more vitamin than is actually required in metabolism—and whole maize can therefore stand depletion of vitamin phosphorus pentoxide to the extent of about one-third before deficiency is likely to be manifested. The following vitamin indices represent determinations on an average series of milling products to an estimated accuracy of about 10 per cent either way:

Product	Whole Maize	Fine Meal	Seconds	Bran	Hominy Chop.	Hominy Samp.
Vitamin Index....	160	120	170	180	380	30

The actual value in any given case depends, of course, upon the mode of milling. The average fine meal is not deficient. The more highly milled high-class table products and breakfast foods are almost invariably notably deficient, and their vitamin indices may vary from the border-line 100 down to 30. Since, however, these products are more expensive, and only used by white people living on a mixed diet, their low vitamin content is of minor importance.

In order to obtain information regarding the relative amount of vitamin *B* contained in various plant tissues, **Osborne** and **Mendel** [52] fed rats upon a vitamin-free basal ration to which was added a weighed quantity of a dried preparation (1 gm.) of the material under investigation. The animals were normal in health and devel-

[52] **Osborne and Mendel,** J. Biol. Chem. 30, 29, 1919; *Ib.* 41, 451, 1920.

opment at the beginning of the experiment and observations were made as to whether the diet received was sufficient to maintain them satisfactorily and induce normal growth. As the daily allowance of *B* was fixed, while the food intake varied with the individual and still more with the character of the plant product fed, the ratio of the latter to the total food eaten varied in general from 10 per cent of the food or less in the case of those growing best to 15 per cent in the case of those growing most poorly.

Alfalfa and clover surpassed all the other vegetable products tested in equal doses, 1 gm. daily as the sole source of *B* being much more efficient than as much as 16 c. c. of milk per day when given in addition to the same basal ration. Several animals gained 175 gm. in eight weeks on this allowance, a growth equal to the best observed in their stock colony. Timothy hay gave good growth at first, but loss of weight resulted after two or three weeks. Hay made from immature clover, timothy or alfalfa contains much more vitamin than does that from the mature plants, which suggests a possible advantage in substituting hay made from less mature plants than ordinarily used. Tomato is rich in the water-soluble vitamin, 1 gm. daily promoting good growth when the animals would eat that amount; even 0.2 gm. occasionally promoted limited growth, or at least maintenance. Spinach, cabbage, turnip and carrot were not unlike in their effect as a source of *B*, 1 gm. doses being no more efficient than 0.5 gm. of alfalfa and clover.

In an earlier paper the same investigators [53] reported that 10 per cent of dried spinach supplies somewhat less than enough of the water-soluble vitamin needed for normal growth; it is about one-quarter as efficient as dried yeast in this respect and twice as efficient as whole wheat, soy beans, dried eggs or milk solids. Fifteen per cent of dried cabbage affords somewhat less than the minimum of water-soluble vitamin needed for normal growth and is thus somewhat less efficient than spinach. Beet root did not equal the other roots tested. The potato is as rich in the water-soluble vitamin as some of the roots tested but even 2 g. of peeled potato did not promote growth at the normal rate during the entire eight weeks period. Dried potato peel is no richer in vitamin content than equal amounts of whole potato. There is no noteworthy difference between new and old potatoes as regards water-soluble vitamin content.

The bulb of the onion also contains considerable quantities of *B*. The variability in content of *B* shown by tissues of the same type is demonstrated by the observations of **Steenbock** and **Gross** [54] that

[53] **Osborne and Mendel, J. Biol. Chem. 37, 187, 1919.**
[54] **Steenbock and Gross, J. Biol. Chem. 40, 501, 1919.**

sufficient of *B* to permit growth was furnished by 15 per cent of carrot, dasheen, or rutabaga, and by a somewhat larger quantity of sweet potatoes, but even 25 per cent of the ration in the form of sugar beet or mangel gave no evidence of furnishing this factor.

Fruits may serve to provide a certain amount of *B*, though they cannot be regarded as rich in this factor. Oranges, lemons, grapefruit, and prunes are richer, grape-juice, apples and pears poorer in this respect.[55] The banana appears to be almost, if not entirely deficient in *B*.[56]

Of the various animal products eggs are quite satisfactory both for growth and for protection against polyneuritis. Milk is not a good source of *B*, although different milks may vary, probably vary considerably, according to the proportion of *B* present in the diets of the lactating animals.[57] Beef muscle contains a comparatively small quantity of *B*, which is readily extracted from it by water. Liver tissue was found to be much richer, as were also the proteins of heart, kidney, and brain tissue,[58] and the pancreatic gland.[59] **Swoboda,**[60] who used the yeast test (see p. 35), reports that *B* is present in large quantities in most of the organs of internal secretion which are of developmental importance, but that tissues high in nuclear material, such as thymus and lymph gland, are low in vitamin content. Fish muscle has no appreciable amount of *B*.[61]

The accompanying table, showing the comparative relation between certain foodstuffs with regard to their content of antineuritic factor, was compiled by the Medical Research Committee (London) and is based upon the observations of **Chick** and **Hume** and of **Cooper.**[62] Wheat germ was taken as a standard and rated at 100.

Substance	Value	Substance	Value
Rice Germ	200	Peas	40
Wheat Germ	100	Wheat Bran	25
Lentils	80	Beef muscle	11
Yeast (pressed)	60	Potatoes	4.3
Egg yolk Ox liver	50		

[55] **Osborne and Mendel,** J. Biol. Chem. 42, 465, 1920.

[56] **Suguira and Benedict,** *Ib.* 36, 171, 1918; **Loeb and Northrop,** *Ib.* 27, 309, 1916; **Northrop,** *Ib.* 30, 181, 1917.

[57] **McCollum, Simmonds and Pitz,** J. Biol. Chem. 26, 35, 1916. See also **Osborne and Mendel,** J. Biol. Chem. 41, 515, 1920.

[58] **Mendel and Osborne,** Proc. Soc. Exp. Biol. Med. 15, 71, 1918; J. Biol. Chem. 32, 309, 1917; *Ibid.* 34, 17, 1918.

[59] **Eddy,** J. Biol. Chem. 27, 113, 1916.

[60] **Swoboda,** J. Biol. Chem. 44, 531, 1920.

[61] **Drummond,** J. Physiol. 52, 103, 1919.

[62] **Cooper,** Sp. Rept. 38, Med. Res. Com.

The value of fresh fruits and vegetables as antiscorbutics was known long before the rôle of the vitamins or even the existence of such factors was recognized. **Lind** in a treatise on scurvy published in London in the middle of the Eighteenth Century records the remarkable efficacy of orange and lemon juice in the treatment of this disease, and these fruits are still ranked among the most powerful antiscorbutics. Next to oranges and lemons he esteemed cider. **Davis** [63] has investigated commercial pasteurized cider and finds it of some value although low in antiscorbutic content. Fresh lime juice seems to contain only about one-quarter as much *C* as lemon juice, and preserved lime juice none at all.[64] The popular impression that lime juice is potent in the prevention or cure of scurvy is due to a misapprehension, the " lime juice " which earned this reputation having been in fact lemon juice. When the juice of the lime was substituted for this so-called " lime juice " in the stores issued to the navy cases of scurvy became frequent. Grape juice is said to be about one-tenth as efficient as orange juice [65] and banana is effective if taken in rather large amount,[66] but prunes seem to have no antiscorbutic power.[67] Tomatoes are most efficient sources of *C*. **Hess** [68] recommends them as even more serviceable than orange juice in infant feeding. The distribution of *C* in fruits seems therefore to be most unequal.

Dry cereal grains and seeds have always been regarded as free from the vitamin *C*, and rations made up of such components have been used repeatedly to induce scurvy in guinea pigs. **Davis** has made certain observations which seem to indicate that a dry seed ration may actually confer some protection against scurvy. This ration consisted of Kaffir corn, split peas, barley, corn, oats and hay. On this diet, rabbits continued in good condition for 300 days, when th experiment was discontinued. Although twelve litters were born only one litter of five and three young out of five of another were reared. The same ration delayed the onset of scurvy in guinea pigs about 20 days. This appears to be the first report of protection by dry seeds. With five grams of raw dried beans, four guinea pigs received slight protection against scurvy.

The fact that the antiscorbutic factor in seeds is much increased when the seed germinates was observed by **Furst** [69] and has since

[63] **Davis**, private communication.

[64] **Chick, Hume** and **Skelton**, Lancet, 1918, ii., 735.

[65] **Chick** and **Rhodes**, Lancet, 1918, ii., 774.

[66] **Lewis**, J. Biol. Chem. 40, 91, 1919.

[67] **Hess** and **Unger**, J. Biol. Chem. 435, 479, 1918.

[68] **Hess**, N. Y. State Jour. Med. 20, 209, 1920; J. Am. Med. Ass. 75, 568.

[69] **Furst**, Norsk, Mag. Lagev. 1912, 1; Zeit, Hyg. Infek. 72, 121, 1912.

been confirmed by many observers. **Chick** and **Delf**[70] report that the antiscorbutic value of these seeds (dried peas and lentils) after soaking in water for 24 hours and germinating for 48 hours at room temperature is five to six fold that of the dry seeds; while inferior to that of orange and lemon juice, cabbages, or swedes, it is equal to that of many other vegetables such as green beans or potatoes, and superior to that of carrot or beetroot. That the content of *C* in dried beans is notably increased on germination is indicated by **Wiltshire's** observation that germinated beans are more effective than lemon juice as antiscorbutic water[71] although certain observers have reported these less satisfactory than other germinated seeds.[72]

Weill, Mouriquand, and **Peronne**[73] question the efficacy of sprouted cereals as antiscorbutics, but their evidence is not very convincing in view of the well attested success which has accompanied the administration of sprouted seeds in cases of scurvy.

Dyke[74] states that beer made from germinated grain is antiscorbutic, a statement which accords with the belief of the early explorers who used malt or spruce beer or sweetwort (an infusion prepared by pouring boiling water over malt and letting it stand for three or four hours and then using at once) as antiscorbutics. Sweetwort is described in **Cook's** "Voyages" as one of the best of antiscorbutic sea medicines known, if used in time. The "high-dried kilned malt" used by modern brewers appears to have little, if any, antiscorbutic property.[75] Dyke reports the results of an inquiry into the cause of an outbreak of scurvy among a Kaffir battalion in France. The diet of this community was arranged so as to reproduce as nearly as possible that of the native kraals, including the provision of a native beer brewed from millet, of which large quantities are regularly consumed in Africa. An outbreak of scurvy occurred although this is unknown among the Kaffirs under ordinary conditions. Inquiry brought out the fact that the native beer was always brewed from germinated grain, but that in France the germination had been omitted.

The common vegetables show very considerable variation in their content of *C*. **Davis** found great variation even among different lots of fresh string beans. A litter of three guinea pigs were successfully

[70] **Chick and Delf,** Bioch. J. 13, 199, 1919.

[71] **Wiltshire,** Lancet, 1918, 11, 811.

[72] **Comvie,** Edin. Med. J. 1920, 207; **Stevenson,** J. Roy. Army Med. Corps, 35, 218, 1920.

[73] **Weill, Mouriquand and Peronnet,** C. r. Soc. de. Biol. 81, June 8, 1918.

[74] **Dyke,** Lancet, 1918, ii, 513.

[75] **Smith,** Lancet, 1917, ii, 813.

reared on a ration containing 30 grams per day; 15 grams per day of canned string beans delayed, but did not prevent the onset of scurvy. **Campbell** and **Chick** obtained no protection with guinea pigs on a diet containing 5 g. per day of raw string beans.[76]

The leafy vegetables have considerable protective power, fresh cabbage being pre-eminent in this respect. **Davis** found that 7 g. per day represented the minimum ration of cabbage necessary to keep guinea pigs in satisfactory condition. When reduced to 5 g. per day they lost weight. **Chick** and **Rhodes**[77] reported that as little as 2 g. per day prevented the onset of guinea pig scurvy.

Canned spinach was also found to give very different results when different lots were compared. Of four lots examined two were satisfactory and two were unsatisfactory as a source of *C*.[78]

Of the root vegetables, the swede (yellow turnip) appears to be equal if not superior to all other sources of *C*. **Chick** and **Rhodes**[79] found that only 2.5 c. c. per day of the raw swede juice was required to afford complete protection against scurvy to guinea pigs; 20 c. c. of carrot juice was required to give the same degree of protection, and beetroot was less effective than carrot.

Potatoes, raw or boiled, may serve as an effective antiscorbutic, 10 g. raw[80] or 20 g. cooked for one-half hour at 100[81] giving complete protection to guinea pigs. Report 38 of the Medical Research Committee says:[82]

> Among roots and tubers the potato easily takes the first place in practical importance, not so much because of its intrinsic value, but because owing to its abundance, cheapness, and general acceptability large quantities are regularly consumed. There is no doubt that in northern climates the potato is of the utmost value in preventing scurvy during the winter and spring. Epidemics of scurvy have repeatedly followed failure of the potato harvest, e. g. in Norway in 1904, Ireland in 1847. The outbreaks of scurvy reported in Glasgow, Manchester and Newcastle in the spring of 1917 are doubtless to be attributed to the great scarcity of potatoes at that period.

The Report goes on to say:

> Onions take a position between the more and the less potent vegetables They possess a special importance, however, owing to the ease with which they can be transported, and are much appreciated whether raw or cooked by reason

[76] **Campbell** and **Chick**, Lancet, 1918, ii, 321.
[77] **Chick** and **Rhodes**, Lanc. ii, 774.
[78] **Davis**, private communication.
[79] **Chick** and **Rhodes**, Lancet, 1918, ii, 774.
[80] **Chick**, **Hume** and **Skelton**, Lancet, 1919, ii, 774.
[81] **Givens** and **McClugage**, J. Biol. Chem. 42, 491, 1920.
[82] Med. Res. Com. (London) Report 38, p. 60.

of their flavor. For these reasons they should always be included in rationing soldiers, sailors, or other communities of people at the end of long lines of communication cut off from fresh supplies.

Davis[83] found that while the bulb of the onion contains some *C* it is insufficient as the sole source of this vitamin for guinea pigs, even when fed at the rate of 20 g. per day.

That the inclusion of a small supply of vegetables in the diet is not necessarily a complete safeguard is demonstrated by a case of scurvy reported by **Wiltshire.**[84] Sixteen patients, for a month before admission to hospital, had been under a diet which should have not only prevented but actually cured scurvy. Eleven had received " plenty " of onions daily, and most of them had also had potatoes with rice twice a week and spinach twice a week. No alkali was used in the cooking of these vegetables, but the result observed possibly may have been due to the destruction of the vitamin by excessively long cooking.

Animal products are less satisfactory than vegetables in antiscorbutic power. Cow's milk is effective only when fed in relatively very large amounts. **Curran**[85] reported that one pint of milk daily was insufficient to protect adults from scurvy on a diet deficient in fresh meat and vegetables. **Barnes** and **Hume**[86] found that guinea pigs on a diet of oats and bran required from 100 to 150 c. c. and monkeys from 125 to 175 c. c. of milk per day respectively to give adequate protection. **Hart, Steenbock** and **Smith**[87] observed that absolute protection was secured for guinea pigs on a diet of oats and hay by the addition of 84 c. c. of milk to the diet per day. Probably the antiscorbutic power of milk varies rather considerably according to the nature and quantity of the food eaten by the cow.[88]

Hess and **Unger**[89] report that feeding with whole eggs failed to delay the onset of guinea pig scurvy and showed no curative effect in cases of infantile scurvy.

Fresh meat is not without value, if fed in sufficient amounts. **Lind** observed that green turtle soup had curative power in scurvy cases. **Willcox**[90] asserts that fresh meat is a valuable antiscorbutic,

[83] **Davis,** private communication.

[84] **Wiltshire,** Lancet 1918, ii, 811.

[85] **Curran,** Dub. J. Med. Sc. 7, 83, 1847.

[86] **Barnes** and **Hume,** Bioch. J. 13, 306, 1919.

[87] **Hart, Steenbock** and **Smith,** J. Biol. Chem. 38, 305, 1919.

[88] **Barnes** and **Hume,** Bioch. J. 13, 306, 1919; **Dutcher, Pierson** and **Biester,** J. Biol. Chem. 42, 301, 1920; **Dutcher, Eckles, Dahle, Mead** and **Schaefer,** Sci. 1920, 589.

[89] **Hess** and **Unger,** J. Biol. Chem. 35, 479, 1918.

[90] **Willcox,** Lancet 1919, ii, 979.

and **Stefanson**[91] states that fresh meat in large quantities prevented and cured scurvy in his Arctic expeditions. Many additional cases drawn from the experience of explorers and pioneers are quoted in Report 38 of the Medical Research Committee.[92] This Report draws the conclusion that scurvy can be prevented by the use of fresh meat alone if the ration is large. " The history of Arctic experience is full of such cases. **Dr. Rae**, surgeon to the Hudson Bay Company, in his evidence to the Scurvy Commissioners of 1876, stated that among the inhabitants of that district scurvy was almost unknown. The people subsisted almost entirely on meat, but the amount consumed was upon the following scale: Eight pounds fresh venison daily per man, four pounds per woman, two pounds per child.

" **Nansen** and **Johansen**, after leaving the Fram, spent two months, including the winter of 1895-6, on Frederick Jackson Island in a rudely constructed hut. They remained in good health and free from scurvy although obtaining no lime juice and no fresh vegetables and subsisting mainly on fresh walrus and bear meat preserved by cold.

" **Jackson** and **Harley** describe an interesting incident at Kharborova, Yugor Straits, where six Russian priests arrived in the autumn, attended by a small Russian boy. The priests by their religious vows were prevented from eating the fresh meat available; they subsisted on salt fish and there were no vegetables. In the following May the little boy was found to be the only surviving member of the party, and had buried all his late masters in the snow. He suffered from no religious prohibitions and had fed largely on reindeer meat through the winter. A further instance is provided in the experience of **Scott's** first expedition[93] where an outbreak of scurvy was cured by the inclusion in the dietary of large quantities of fresh seal meat.

" From **Colonel Hehir's** report on the medical history of the siege of Kut it is clear that in this case also British soldiers were protected from scurvy by their regular ration of meat or horse flesh, but the amounts they consumed were considerable, 8 to 20 oz. daily. The Indian troops in Kut, on the other hand, who were vegetarians, suffered severely from scurvy.

" Tinned and preserved meats can be dismissed in a word, as not offering any suitable protection from scurvy. Meat in its fresh condition contains the anti-scurvy factor in comparatively low concentration, and after exposure to the temperature necessary for sterilization it is scarcely possible that any significant anti-scurvy properties should be retained.

[91] **Stefanson**, J. Am. Med. Ass. 71, 1715, 1918.
[92] Med. Res. Com. London, Special Report 38, p. 63.
[93] " Voyage of the Discovery."

"The value of frozen meat is probably intermediate between that of fresh meat and tinned meat and, in any case, is likely to be low, especially if it has been for a long period in the frozen condition."

Wiltshire[94] found that of 132 cases of scurvy whose previous diet was investigated, all had received a ration of frozen fresh meat practically every day, and of 175 other cases very few had received it less than three times a week. In the case of tinned meat the vitamin seems to be completely destroyed by the temperature needed for canning.

Similar results were noted by **Comrie.**[95] **Chick, Hume and Skelton**[96] could detect very little beneficial effect when meat juice was fed to guinea pigs, although 20 cc. per day gave slight protection in some cases. **Dutcher, Pierson and Biester**[97] found that the addition of water extracts of raw lean beef (equivalent to 5, 10, 15 or 20 grams of beef) had no effect on the time of onset of scurvy or on the length of life of the animals. That the beef extract contained no deleterious ingredients was shown by the fact that when orange juice was added to the diet in addition to the beef juice, there was no scurvy and the animals were maintained in excellent condition.

Hughes, Payne and **Fox** have carried on experiments which tend to show that hens supplied with food having a low vitamin content will produce eggs similarly low in vitamins. They further observe that eggs from hens receiving a feed having a low vitamin content yield a smaller proportion of strong, vigorous chicks than eggs from hens receiving a feed well supplied with vitamins.[98]

[94] **Wiltshire,** Lancet 1918, ii, 811.

[95] **Comrie,** Edin, Med. J. 24, 207, 1920.

[96] **Chick, Hume and Skelton,** Bioch. J. 1918, 12, 131; Lanc. 1919, ii. 322.

[97] **Dutcher, Pierson and Biester,** J. Biol. Chem. 42, 301, 1920.

[98] Am. Food. Jour., Oct. 1921.

CHAPTER V

THE STABILITY OF THE VITAMINS WITH REGARD TO CONDITIONS OF HEATING, DRYING, AND STORING.

The effect of heating, drying, and storing upon vitamins is of serious import in connection with the methods used in preparing foods. It is generally conceded that fresh foods are more valuable than those which have been preserved, and that a diet should if possible include a certain amount of uncooked food, but the basis for this belief must be investigated.

The vitamins, which are known to be highly sensitive to reagents, are the constituents of foods which would seem most likely to undergo undesirable alteration during heating and drying. Unfortunately here, as in many other questions connected with the vitamins, authorities are by no means agreed.[1]

Chick and **Hume** and **Davis** have referred to the unsatisfactory character of much of the data given in the literature.[1a] **Chick** and **Hume** in experiments on the effect of temperature on vitamins note that the temperatures given are usually those registered by the autoclave or steamer used, no measurement being made in the interior of the substance heated. This is an important point, as the latter temperature remains for a surprisingly long time far below the former, especially if the material investigated has a low conductivity, which is true of most foodstuffs, particularly if fairly dry. **Davis** remarks that for comparative purposes with cooked food it is important to know how it was cooked, how long, the state of division, the amount cooked at a time, the amount of water used if boiled, the temperature if baked, and the interval between cooking and consumption. The data concerning commercial preserved food is interesting from an economic standpoint but is of little scientific value since the conditions under which is was prepared are not accurately known.

[1] A summary of the factors influencing the vitamin content of foods is given by **Dutcher** (J. Ind. Eng. Chem. 1921, 1102).

[1a] **Chick and Hume**, Proc. Roy. Soc. (London) 90 B, 60. **Davis**, J. Home Econ. 12, 208, 1920.

Vitamin *A* was reported by the earlier investigators as very stable to heat. **McCollum** and **Davis**[2] found it present in the ether extract of boiled egg yolks. **Osborne** and **Mendel**[3] stated that passing live steam through butter-fat for two and a half hours or longer did not destroy its nutritive efficiency, and **McCollum**[4] supported their evidence by the statement that the *A* in butter-fat was not destroyed by heating to 100° for over an hour. A curious fact was noted by **Osborne** and **Mendel**[5] when they separated butter-fat into a high and a low melting portion by fractional crystallization from alcohol. In the butter "oil" in which vitamin *A* is more concentrated, deterioration occurs on keeping, even in absence of light, to such an extent that within a year the potency is almost completely lost, while some of the same lot of butter-fat from which this oil had been prepared was still effective.

Drummond[6] found that low grade whale and cod liver oil which had been prepared by steam digestion of the tissues were still rich in *A* and concluded that the vitamin was therefore thermo-stable. In a later experiment he used a high grade whale oil which had been treated with soda to remove the free fatty acids, and subsequently clarified with fuller's earth. It was rich in *A* as demonstrated by experiments with rats, while a similar sample hardened by the usual process, involving exposure to hydrogen gas at 250° C. for 4 to 6 hours was found entirely deficient in *A*. In order to test whether destruction is brought about by reduction of some unsaturated substance or simply by high temperature the oil was heated to 250° C. for four hours. It proved destitute of *A* after this treatment. Next, samples of whale oil were heated to 100° and 150° for four hours respectively. In both cases destruction of *A* was almost complete. Eventually he concluded that the vitamin in both butter-fat and whale oil[7] was readily destroyed by short exposure (one hour) to 100°. Destruction occurs, but is less rapid, when exposed to temperatures ranging from 50 to 100°. Exposure of oil to a temperature of 37° for several weeks may cause destruction of *A* if contact with air or oxygen is extensive.

Commenting on these results, **Osborne** and **Mendel**[8] remark:

[2] **McCollum** and **Davis**, Proc. Soc. Exp. Biol. Med. 11, 101, 1919.

[3] **Osborne** and **Mendel**, J. Biol. Chem. 20, 381, 1915.

[4] **McCollum**, Am. J. Publ. Health 8, 191, 1918.

[5] **Osborne** and **Mendel**, J. Biol. Chem. 24, 37, 1915.

[6] **Drummond**, J. Physiol. 52, 95, 1918–19.

[7] **Drummond**, Bioch. J. 13, 81, 1919; **Drummond** and **Coward**, *Ib.* 14, 734, 1920.

[8] **Osborne** and **Mendel**, J. Biol. Chem. 41, 557, 1920.

In considering **Drummond's** experiments one is struck by the fact that even with six per cent of unheated butter-fat in the diet his control rats grew at much less than the normal rate. This is contrary to the experience of several investigators, including ourselves, and raises a question as to the value of the untreated butter-fat or the food intake of the animals used by him. At any rate the reason for the discrepancies between us is not apparent.

In confirmation of their earlier experiments they heated dry butter-fat in an air bath at 96° for 15 hours without destroying sufficient *A* (if any) to render the butter-fat noticeably less efficient. They point out, however, that their butter-fat was fed in amounts which were probably well above the minimum and that a certain amount of destruction might therefore have escaped detection.

Steenbock, Boutwell and **Kent** [9] found that 12 per cent of butter-fat which had been heated to 100° for four hours was less efficient for the nutrition of rats than 5 per cent unheated butter-fat incorporated into a similar basal ration. It is to be noted that their experiments showed a very considerable variation in different butter-fats which had been subjected to the minimum heat necessary for separation of the fat. While 5 per cent in the diet was usually sufficient as a source of *A* there were numerous instances in which this amount proved unsatisfactory.

Recent experiments of **Hopkins** [10] appear to demonstrate conclusively that the discrepancies in the results of different observers are due to different degrees of aeration while heating. This hypothesis finds confirmation also in the work of Drummond and Coward (l. c.).

Zilva [11] has observed that ultraviolet rays have a destructive effect upon the vitamin *A* in butter-fat. Butter and cod liver oil exposed for 8 hours to ultraviolet light undergoes a very noticeable change and the *A* in it becomes inactivated. That the slight elevation of temperature is not responsible for this is seen from the fact that addition of butter kept for 6 hours at 35° at once induces growth. On the other hand, the experimental evidence indicates clearly that the inactivation is due to the ozone formed by the action of the ultraviolet rays upon the oxygen of the air. **Zilva** suggests that the same effect would probably be brought about in exposing milk to ultraviolet rays, a point which is worthy of consideration inasmuch as this method has been proposed for sterilizing milk.

Animal tissues (the brain, heart and liver of the pig) which had been dried at 90° were found by **Osborne** and **Mendel** [12] to retain

[9] **Steenbock, Boutwell** and **Kent**, J. Biol. Chem. 35, 517, 1918.
[10] **Hopkins**, Biochem. J. 14, 724, 1920.
[11] **Zilva**, Bioch. J. 13, 165, 1919; 14, 740, 1920.
[12] **Osborne** and **Mendel**, J. Biol. Chem. 34, 17, 1918.

a considerable amount of *A*, but these materials were fed at a high level so that a small amount of destruction would not necessarily have been detected.

Steam-treated palm kernel oil is recommended as a control fat by **Stammers.**[13] By passing steam through this oil for three or four hours at a temperature of 230° to 260° C. the oil is rendered quite free from vitamin *A*.

Steenbock and **Boutwell**[14] found *A* as it occurs in a grain, in leaf and stem tissue, in fleshy roots, and in a cucurbitous vegetable like squash, to be comparatively stable at high temperature. Treatment consisting of autoclaving for three hours at 15 pounds pressure (120° C.) does not destroy any of the *A* in yellow corn, nor does it cause any noticeable destruction of the *A* in chard, carrots, sweet potatoes, or squash as demonstrated when these vegetables were fed in amounts varying from 5 to 15 per cent of the ration. If some destruction occurred it was not detected, but with the amount fed the investigators believe that it could not have occurred to any considerable degree, otherwise normal growth or long continued growth with reproduction would not have been possible. In the case of alfalfa the data was not decisive, but it appears that the destruction, if any, was too little to induce deficiency in the ration. No deleterious action of the ensiling process on *A* could be demonstrated in a short-time experiment.[14a]

Sure, Barnett and **Read**[15] report that the vitamin *A* present in the Georgian velvet bean is stable after autoclaving for one hour at 15 pounds pressure.

With regard to the heat stability of the *A* present in green cabbage leaves, **Delf**[16] reported that heating for a half hour at temperatures from 100 to 120° caused slight though perceptible deterioration. After heating at 130° for two hours, however, a serious amount of destruction had taken place. **Delf** and **Skelton**[17] estimated that 86 per cent of the *A* in cabbage was lost when the cabbage was dried at a low temperature and stored for two weeks. **Osborne** and **Mendel**[18]

[13] **Stammers,** Biochem. J. 15, 489, 1921.

[14] **Steenbock** and **Boutwell,** J. Biol. Chem. 41, 163, 1920.

[14a] Fat-soluble *A* is not destroyed, according to **Steenbock, Sell** and **Buell,** by treatment in the proportion of 1 gm. of fat to 2 c. c. of 20% alcoholic potash at 37° C. for 4 hours and for 30 minutes at the boiling temperature. (Steenbock, Sell and Buell, J. Biol. Chem. 47, 89, 1921).

[15] **Sure, Barnett** and **Read,** Proc. Soc. Biol. Chem. J. Biol. Chem. 46, li, 1921.

[16] **Delf,** Bioch. J. 12, 416, 1918.

[17] **Delf** and **Skelton,** Bioch. J. 12, 448, 1918.

[18] **Osborne** and **Mendel,** J. Biol. Chem. 41, 557, 1920.

claim, however, that these experiments lack adequate controls and are not convincing.

That drying does not necessarily bring about destruction of *A* is demonstrated by the successful experiments of **Osborne** and **Men-**

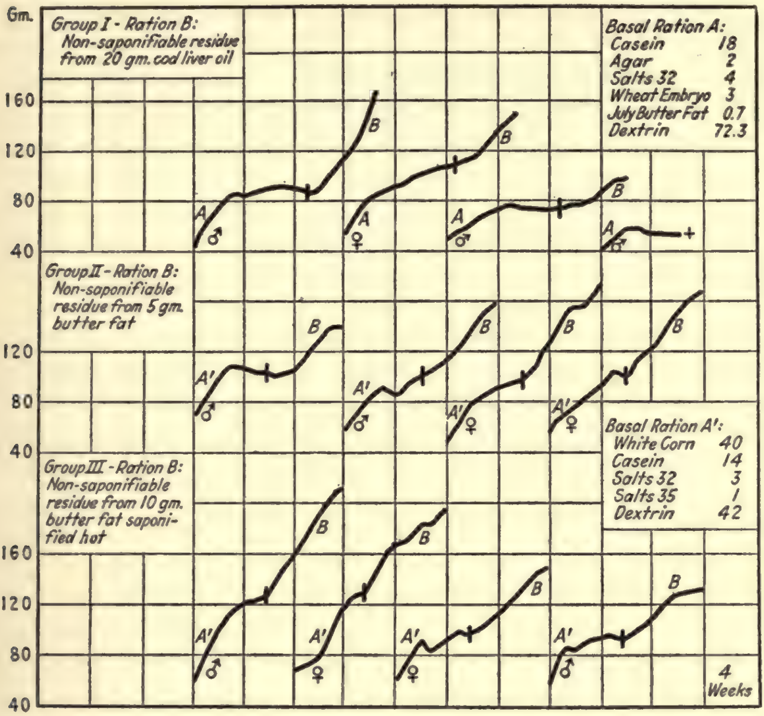

FIG. 4.—The resistance of the fat-soluble vitamin to destruction by saponification of the fats in cod liver oil and butter-fat is indicated by the above curves showing the prompt recovery of growth in rats when an ether extract of a solution of the soaps was added to the basal rations.

Courtesy of Prof. H. Steenbock, M. T. Sell, M. V. Buell and Journal of Biological Chemistry (47, 109, 1921).

del[19] with rats on a diet including only 0.1 g. of tomato which had been dried at 60° as the sole source of *A*. **McCollum**[20] discounts the effect of heat on this vitamin entirely, asserting that there is not the slightest danger of destroying the growth-promoting substances in fruits and vegetables by the ordinary canning processes. **Campbell**

[19] **Osborne and Mendel**, J. Biol. Chem. 41, 549, 1920.

[20] **McCollum**, Am. J. Pub. Health, 8, 191, 1918.

and **Chick** have reported,[21] however, that canned "green-leaf vegetables" are lacking in *A* unless the liquor is included in the portion eaten.

As a result of his earlier work, **Drummond** was of the opinion that the destruction of *A* at high temperatures could be neither an oxidation nor a hydrolytic process. Later evidence, however, led him to agree with **Hopkins**[22] that this is an oxidation process.[23] On the other hand, **Steenbock, Sell, Nelson,** and **Buell**[24] have recently reported that the extract of *A* obtained from alfalfa is not destroyed by oxidation with hydrogen peroxide or by reduction with nascent hydrogen.

Commenting on the lack of uniformity in the observations reported from different sources, **Steenbock** and **Boutwell**[25] conclude that the data of the different investigators in this field are substantially true to fact and that the variance of opinion is due to a failure to appreciate that the reaction of destruction may be one of low velocity impeded or accelerated by secondary factors. For example, in a ration containing twice as much vitamin as necessary for normal growth no evidence of its destruction would of course be obtained even though one-half of the amount present might have been destroyed by the treatment. In addition, there is no doubt, according to **Steenbock** and **Boutwell,** that there obtains a great difference in the stability of the vitamin as found in different materials.

The antineuritic vitamin appears to be somewhat less stable than the vitamin *A*. Its presence in dry grains and other dried foodstuffs shows that it can withstand desiccation, and there is much evidence to indicate that heating at the temperature and for the length of time ordinarily employed in the cooking of food produces comparatively little if any destruction of the antineuritic properties, but at temperatures above 100° C. the rate of destruction appears to increase rapidly. Observations to this effect were recorded by some of the earlier observers, although their experiments were not carried on quantitatively. **Grijns**[26] showed that one-half hour's exposure to 120° C. destroyed the antineuritic power of unmilled rice and "Katjang idjo" beans. **Eijkman**[27] found that seeds which had been

[21] **Campbell and Chick,** Lancet 1919, ii, 320.

[22] **Hopkins,** Brit. Med. J. 1919, ii, 147.

[23] **Drummond and Coward,** Biochem. J. 14, 661, 1920.

[24] **Steenbock, Sell, Nelson, and Buell,** Proc. Soc. Biol. Chem. J. Biol. Chem. 46, XXXII, 1921.

[25] **Steenbock and Boutwell,** J. Biol. Chem. 41, 164, 1920.

[26] **Grijns,** Geneesk, Tydschrift. *v.* Ned. Ind. 1901.

[27] **Eijkman,** Arch. f. Hyg. 58, 150, 1906.

heated from 115 to 135° for two hours had little protective action against polyneuritis. **Holst**[28] reported that dried peas and unpeeled barley did not lose their antineuritic power on heating to 115° for half an hour. **Weill** and **Mouriquand**[29] found that barley, rice and maize which had been heated for an hour and a half at 120° no longer possessed any antineuritic property, and **Emmett and Luros**[30] report that while heating for 120° for one hour had no effect on the antineuritic power of unmilled rice it was partly destroyed at the end of two hours and completely destroyed in six hours, and that the vitamin was still less stable to heat after it had been extracted from its natural source. Curiously, rice which has completely lost its antineuritic power was still able to promote the growth of young rats. **Braddon**[31] states that persons eating parboiled rice did not contract beriberi provided the pericarp had not been removed by one boiling.

In a carefully conducted experiment by **Chick** and **Hume**[32] the antineuritic property of wheat embryo as tested by its curative effect on polyneuritic pigeons was unaffected by heating to 100° C. for two hours; after 40 minutes at 113° its curative effect was reduced by one-half, and after two hours at 118° to 124° the antineuritic activity was less than one-fourth that of untreated material.

McCollum and **Davis**[33] reported that heating to 120° for one hour did not injure the growth-promoting properties of wheat embryo, but their results are open to the criticism that the wheat embryo was fed at too high a level (13 per cent of the diet) to show whether partial destruction had taken place, since they themselves had found that two per cent of unheated wheat embryo was sufficient to induce normal growth. **McCollum, Simmonds,** and **Parsons**[34] found that peas which had been cooked under 15 pounds pressure for an hour and a quarter and then dried in a current of hot air when fed to the extent of 25 per cent of the diet provided enough *B* to enable young rats to grow at normal rate to full adult size, but here again the amount fed was probably far above the minimum and considerable destruction may have escaped notice.

Miller,[35] using the **Williams** yeast test for *B*, calculated that by boiling navy beans for 30 minutes at 120° a loss of 40.6 per cent of

[28] **Holst,** J. Hyg. 7, 619, 1907.
[29] **Weill** and **Mouriquand,** C. r. soc. biol. 78, 649; 81, 432, 1918.
[30] **Emmett** and **Luros,** J. Biol. Chem. 43, 265, 1920.
[31] **Braddon,** "The Cause and Prevention of Beriberi." London, 1907.
[32] **Chick** and **Hume,** Proc. Roy. Soc. 90 B, 66.
[33] **McCollum** and **Davis,** J. Biol. Chem. 23, 191, 247, 1915.
[34] **McCollum, Simmonds** and **Parsons,** J. Biol. Chem. 37, 287, 1919.
[35] **Miller,** J. Biol. Chem. 44, 159, 1920.

the growth-promoting power was brought about and that practically the same loss (37.5 per cent) was brought about by boiling in 0.5 per cent sodium bicarbonate solution for one hour and ten minutes. **Davis** [36] found that seeds suffered a complete loss of *B* on heating for two and a half hours to 120° C., although at the end of 35 minutes at this temperature there was sufficient vitamin left to keep pigeons in good condition for 280 days.

It seems clear, therefore, that so far as grains and pulses (peas and beans) are concerned the *B* vitamin is unquestionably destroyed by heating to 115° to 120° for one to two hours, while deterioration is much slower at 100° and may therefore be inconsiderable in the course of ordinary cooking.

Yeast is affected in much the same way, a temperature of 120° bringing about complete destruction of the vitamin content in two hours, while 100° seems to be ineffective.[37] The yeast vitamin seems to be less stable when separated from the yeast plant than when *in situ,* since **Myers** and **Voegtlin** [38] found that the active precipitate from autolyzed yeast becomes inactive on drying at room temperature. In this connection it may be noted that **Brill** found the vitamin content of hydrolyzed extract of rice polishings to deteriorate somewhat with age.[39]

Karr [40] states that drying at 100° C. does not affect the antineuritic efficiency of yeast, tomatoes or milk, but autoclaving at 120° C. for three to four hours leads to some destruction.

Miller (loc. cit.) found that carrots when tested by the **Williams** method had apparently suffered no loss of *B* when heated to 115° for 45 minutes. **Whipple,**[41] using a modification of the same method, reported that cabbage had lost some but not all of its *B* on boiling for 30 to 60 minutes at 100° C. **Davis** [42] states that while eight grams of raw potato supplies sufficient antineuritic to keep pigeons in good condition for 590 days an equal amount of either boiled or baked potato failed to give complete protection. Two grams of commercial dried potato gave no protection, but four grams had some protective power; though the experiment was not carried to its conclusion.

[36] **Davis,** J. Home Ec. 12, 209, 1920.

[37] **Chick** and **Hume,** J. Roy. Med. Corps, 20, 121, 1917; **Emmett** and **Luros,** Proc. Soc. Biol. Chem. J. Biol. Chem. 41, vii, 1920.

[38] **Myers** and **Voegtlin,** J. Biol. Chem. 42, 199, 1920.

[39] **Brill,** Phil. J. Sci. 12, 199, 1917.

[40] **Karr,** J. Biol. Chem. 44, 255, 1921.

[41] **Whipple,** J. Biol. Chem. 44, 175, 1920.

[42] **Davis,** J. Home Ec. 12, 209, 1920.

With regard to the antineuritic vitamin found in animal products, **Grijns**[43] noted that exposure for one-half hour to 120° C. destroyed the antinueritic factor of buffalo meat, and **Holst**[44] found some loss of antineuritic power in beef after heating to 110° C. for half an hour. The destruction was more marked after exposure to 120° C. for an hour. **Schaumann**[45] induced polyneuritis in dogs by a diet of horse flesh heated from one to three hours at 120° to 130° C., but **Eijkman**[46] did not succeed in destroying the antineuritic power of horse flesh by heating to 120° C. for two hours.

According to **Weill, Mouriquand** and **Michel**[47] while an exclusive diet of raw, frozen or salted meat failed to produce nervous disorders due to nutritional deficiency, cats which had been given an exclusive diet of cooked meat succumbed with symptoms of inanition. Sterilized meat caused the appearance of nervous disorders after 35 to 39 days. Aging of the sterilized meat appeared to reduce the time required for such manifestations. Meat which had been sterilized and then kept 14 months caused nervous manifestations due to nutritional deficiency after 25 to 35 days. The symptoms were similar to, if not identical with, those produced in pigeons by feeding decorticated cereals.

Milk, at best, is not rich in the antineuritic factor and prolonged heating probably has a deleterious effect upon it. **Stepp**[48] suggested that the nutritive value of milk was somewhat lessened by boiling as it was necessary to use rather more boiled milk than raw to supplement a diet of bread which had been extracted with alcohol, but it is open to question whether this is due to destruction of the vitamin or to some other effect.[49] **McCollum** and **Davis**[50] found that whey from which the casein and albumin have been removed still possesses growth-promoting properties after heating to 120° for six hours, and would induce normal growth when fed at high level, and **Osborne** and **Mendel**[51] were led to conclude from their own observations that: "We have no reason to believe that the nutrition-promoting properties of milk are lost by brief periods of heating." That prolonged

[43] **Grijns**, Geneesk, Tidjschrift *v.* Ned. Int. 1901.

[44] **Holst**, J. Hyg. 7, 619, 1907.

[45] **Scnaumann**, Arch. f. Schiffs. Trop. Hyg. Beihefte 3, 14, 1910.

[46] **Eijkman**, Arch. f. Hyg. 58, 150, 1906.

[47] **Weill, Mouriquand** and **Michel**, Compt. rend. soc. biol. 79, 189, 1916.

[48] **Stepp**, Ztsch. Biol. 1911, 57, 135.

[49] See Ch.

[50] **McCollum** and **Davis**, J. Biol. Chem. 23, 247, 1915–16; **Funk** and **Macallum**, J. Biol. Chem. 27, 51, 1916; **Emmett** and **Luros**, *Ib.* 38, 257, 1919.

[51] **Osborne** and **Mendel**, J. Biol. Chem. 34, 541, 1918.

heating at a high temperature may, however, cause serious deterioration of milk with respect to its antineuritic power is suggested by the work of **Emmett** and **Luros**[52] who separated the vitamin from protein-free milk with fuller's earth[53] and used this in the treatment of polyneuritic pigeons. While 0.6 g. of the unheated material was unfailingly successful in promoting a cure, an equal amount of material which had been heated for six hours at 120° C. had no noticeable effect.

According to **Chick** and **Hume**[54] little antineuritic factor is likely to be present in canned and sterilized foods. **Daniels** and **McClurg**[55] disputed this on the evidence of their experiments in which normal growth was secured with young rats on a diet in which the only source of *B* was in the form of vegetables (soy or navy beans or cabbage) which had been cooked in a pressure cooker at 120° C. for 20 minutes, boiled in distilled water for 45 minutes, or boiled in a dilute alkali for 30 minutes. The various methods of treatment are said not to have appreciably affected the vitamin content. Their results are vitiated, however, by the fact that the heated vegetables were fed in large excess, the equivalents of 50 g. navy beans, 55 g. of soy beans, or 250 g. of cabbage for every 100 g. of dry food. **McCollum, Simmonds** and **Pitz**[56] found 25 g. of navy beans per 100 g. of dry food sufficient to supply the vitamin needed for normal growth, so the vitamin content of the vegetables might have been reduced by one-half in the above experiments without detection.

The effect of radium emanation on the vitamin *B* in yeast has been studied by **Funk**[57] and by **Suguira** and **Benedict.**[58] **Funk** reported no loss of activity in the yeast extract with regard to either antineuritic or growth-promoting effect, after exposure to radium, but **Suguira** and **Benedict** disagree with him concerning the growth-promoting power, claiming that this factor may be partially inactivated by exposure to radium emanation. The reason for this discrepancy is not apparent.

The antiscorbutic factor is the most sensitive of the three vitamins to heat and drying. The failure of dried vegetables and herbs and preserved meats and fish to prevent or cure outbreaks of scurvy has

[52] **Emmett** and **Luros**, J. Biol. Chem. 43, 265, 1920.
[53] **Seidell**, Bull. Hyg. Lab. U. S. P. H. 1916, 31, 364.
[54] **Chick** and **Hume**, J. Roy. Med. Corps, 29, 121, 1917.
[55] **Daniels** and **McClurg**, J. Biol. Chem. 37, 201, 1919.
[56] **McCollum, Simmonds** and **Pitz**, J. Biol. Chem. 29, 521, 1916.
[57] **Funk**, Proc. Soc. Exp. Biol. Med. 14, 9, 1916.
[58] **Suguira** and **Benedict**, J. Biol. Chem. 39, 421, 1919.

been demonstrated repeatedly in the naval history of every country.[59] **Stefanson**[60] has asserted on the basis of his Arctic experience that the antiscorbutic properties of fresh foods are diminished or entirely lost in the course of any of the ordinary methods of preservation, canning, pickling, drying, etc. **Chick** and **Hume**[61] assert that all dried foodstuffs, including desiccated vegetables examined by them, are more or less deficient in antiscorbutic vitamin. At first they thought the temperature at which the tissues were dried seemingly was a matter of indifference, but later work indicates this statement to be too sweeping.

The partial or complete loss of *C* during heating has been recorded by many observers working with many different foodstuffs. Cabbage, which is rich in *C* in the raw state, has been a favorite subject for experimentation. **Campbell** and **Chick**[62] found that heating in water for about one hour at 90° to 100° C. caused a loss of about 70 per cent of the antiscorbutic power of cabbage, and state that further loss may be expected to take place if the canned material is stored for any length of time before feeding. **Delf**[63] estimates that about 70 per cent of the original value of cabbage is lost after one hour's heating to 60° C., and over 90 per cent after the same period at 90° C. Twenty minutes heating at 90° to 100° C appears to have practically the same effect as heating for an hour at 60° C. In these experiments a small amount of cabbage was added to a diet of grain and autoclaved milk which had been found to produce scurvy when fed alone. When the autoclaved milk was omitted and the cabbage ration was increased to from 15 to 30 g. the destructive effect of heat was less noticeable, even when the cabbage was heated to 100° to 130° C for one to two hours. **Delf** suggests that this may be due to the fact that in the later experiments with the larger ration both *A* and *C* were drawn from the same source. Much evidence has been accumulated by the group of workers at the Lister Institute of Preventive Medicine, London, pointing to a distinct and separate action of these two factors in the nutrition of young guinea pigs. **Tozer** has, however, found that a lack of growth fat-soluble factor in the diet is accompanied by a series of changes in the histology of the rib-junction which is almost indistinguishable from that caused by a slight deficiency of antiscorbutic factor. It is therefore not impos-

[59] See Ch. XII.

[60] **Stefanson**, Jour. Am. Med. Ass. 71, 1715, 1918.

[61] **Chick** and **Hume**, Tr. Soc. Trop. Med. Hyg. 10, 141, 1917.

[62] **Campbell** and **Chick**, Lancet, 1919, ii, 320.

[63] **Delf**, Bioch. J. 12, 416, 1918.

sible that the antiscorbutic value of a diet may be enhanced and may show greater heat stability when the antiscorbutic factor and the growth fat-soluble factor are derived from the same foodstuffs.

Holst and **Frölich**[64] found that freshly expressed cabbage juice lost its antiscorbutic power after heating from 60° to 100° C. for ten minutes. The destruction was not so complete if the juice was acidified with 0.5 per cent of citric acid before heating. **Delf**[65] failed to confirm this result with acidified cabbage juice, and **Holst** and **Frölich** themselves found that the presence of acid did not prevent the destruction of the *C* in dandelion or sorrel juice.

According to **Givens** and **Cohen**[66] cabbage which has been heated in an oven for two hours at 75° to 80° C., then dried at 65° to 70° C. for several days loses its antiscorbutic power completely. Cabbage which has been cooked for 30 minutes, then subjected to drying for two days at 65° to 70° is likewise devoid of antiscorbutic power.

Ellis, Steenbock and **Hart**[67] report that desiccation at 65° C. even in an atmosphere of carbon dioxide causes destruction of the antiscorbutic in cabbage, which indicates that the destructive agency in this case is heat rather than an oxidation. On the other hand, the same experimenters find that oxidizing agents will destroy the *C* in orange juice, although it is not affected by aeration.[67a]

With regard to other vegetables there are fewer observations, but the general trend is the same. **Campbell** and **Chick**[68] found that string beans which had been heated to 90° to 100° C. for an hour on two successive days had lost about 90 per cent of their content of *C*. **Chick** and **Delf**[69] note that a considerable amount of the antiscorbutic power generated in dried peas and lentils on germinating is destroyed by boiling, and that the cooking of these should therefore be as short as possible. **McClendon, Cole, Engstrand,** and **Middlekauf**[70] assert that heating at 70° C. gelatinizes the starch without destroying the *C* in sprouted cereals. They base this statement on the fact that a guinea pig lived for 34 days without showing scorbutic symptoms on an exclusive diet of sprouted cereals which had been heated to this temperature.

[64] **Holst** and **Frölich,** Ztschr. Hyg. 72, 1, 1912.

[65] **Delf,** Bioch. J. 12, 416; 14, 211.

[66] **Givens** and **Cohen,** J. Biol. Chem. 36, 127, 1918.

[67] **Ellis, Steenbock** and **Hart,** J. Biol. Chem. 46, 367, 1921.

[67a] See also the work of **Sherman, LaMer** and **Campbell** referred to on p. 102.

[68] *Loc. cit.*

[69] **Chick** and **Delf,** Bioch. J. 13, 199.

[70] **McClendon, Cole, Engstrand** and **Middlekauf,** J. Biol. Chem. 40, 243, 1920.

Delf [71] found that swede juice was more stable to heat than cabbage juice, although the vitamin content is partially destroyed by heating to 130° C.

Denton and **Kohman** [72] could detect no loss of *C* in carrots which had been cooked in the ordinary manner or canned by the cold-pack method, but their experiments were carried on with rats, a species of animals which are not susceptible to scurvy. **Hess** and **Unger**,[73] on the other hand, found considerable loss of *C* on boiling carrots for 45 minutes, as demonstrated with guinea pigs fed on 35 g. of carrots per day added to a basal ration of hay, oats and water *ad lib*. These investigators noted a great variation in the nutritive value of old and young carrots, and suggest that a similar difference exists between old and young specimens of various other vegetables as well, a variability which makes it very difficult to prepare a table of the comparative antiscorbutic value of different foods. Old carrots have a two-fold disadvantage in that they contain less antiscorbutic body than young vegetables and that they require additional boiling, which still further decreases their limited supply of this food factor.

Tomatoes appear to retain their antiscorbutic properties better than do many other vegetables, although some destruction undoubtedly takes place. According to **Givens** and **McClugage**,[74] a daily supplement of 2.5 g. of fresh raw tomatoes will protect a guinea pig from experimental scurvy on a diet which is adequate in other respects but devoid of any other source of *C*. When the tomatoes are heated at 100° C. for 15 minutes or longer a larger daily dosage must be supplied to insure protection against this disease. Tomatoes canned according to the usual commercial method, heating under five pounds pressure for 10 minutes, will prevent scurvy in a guinea pig when fed in daily portions of 10 g. and this same amount proved sufficient for protection when the heating was increased to 30 minutes at 15 pounds pressure. **Hess** and **Unger** [75] found 5 c. c. of canned tomatoes per day sufficient to protect guinea pigs against scurvy, and in some cases even a less amount than this was adequate. If the tomato is boiled **Hess** and **Unger** likewise found that this vegetable loses somewhat in efficacy, although 5 c. c. of it was still effective as protection. In further experiments it was found that pigs receiving 10 c. c. of canned tomato daily throve as well as those which received

[71] **Delf**, Bioch. J. 14, 211.

[72] **Denton and Kohman**, J. Biol. Chem. 36, 249, 1918.

[73] **Hess and Unger**, J. Biol. Chem. 38, 293, 1919.

[74] **Givens and McClugage**, Proc. Soc. Biol. Chem., J. Biol. Chem. 41, xxiv, 1920.

[75] **Hess and Unger**, J. Biol. Chem. 38, 293, 1919.

60 c. c. It was found also that pigeons suffering from polyneuritis could be cured by giving them 5 c. c. daily of this foodstuff.

Hess has arrived at the interesting conclusion that the juice of canned tomatoes is one of the most valuable sources of vitamins. In most cases fruit or vegetable juices contain one or at most two vitamins while in the tomato are present all three, *A*, *B* and *C*. On the basis of lower cost Hess recommends the juice of canned tomato in preference to orange juice in the feeding of infants. An ounce per day is sufficient for the average child.[75a]

Sherman, LaMer and **Campbell** [76] studied the time curve of the destruction of *C* in filtered canned tomato juice, and concluded from the low temperature coefficient and the colloidal nature of the material that the reaction, in this case at least, is of the heterogeneous type, with diffusion playing an important rôle. Oxidation does not appear to be an important factor.[76a]

Chick and **Rhodes** [77] found 20 g. per day of cooked potato necessary in order to protect guinea pigs against scurvy. **Givens** and **McClugage** [78] fed potatoes, raw, cooked and dried under varying

[75a] Am. Food. Jour. Oct. 1921.

[76] **Sherman, LaMer** and **Campbell,** Sci. Aug. 26, 1921, 176.

[76a] The effect of temperature and of hydrogen-ion concentration upon the rate of destruction of antiscorbutic vitamin in tomato juice has been considered by **LaMer, Campbell** and **Sherman** (Proc. Soc. Exptl. Biol. Med. **18**, 122, 1920). Feeding experiments were conducted upon guinea pigs fed a basal diet containing optimum amounts of all nutrients except vitamin *C*. This was supplied exclusively in the form of filtered canned tomato juice. Relative amounts of vitamin in treated and untreated portions of juice were measured by the amounts required to prevent scurvy or by a rating of the severity of the scurvy produced. Boiling tomato juice of natural acidity p_H 4.2 for one hour destroyed 50 per cent of its antiscorbutic vitamin; four hours' boiling destroyed 70 per cent. Juice neutralized in whole or in part, boiled one hour, cooled and reacidified at p_H 5.1 to 4.9 (natural acidity less than half neutralized) shows a destruction increased to 58 per cent. By neutralizing a larger proportion of the neutral acidity, the rate of destruction of the vitamin at 100° regularly increased. Juice to which alkali was added at an initial p_H of 11, which fell to about 9 during the hour of heating, was immediately cooled and reacidified. By feeding such treated juice it was shown that destruction increased to 65 per cent. On repetition of the latter experiments without reacidification and the treated juice stored up to five days in the refrigerator before feeding showed 90 to 95 per cent destruction. It was uncertain whether the difference between reacidified and non-reacidified juices is due wholly to prolonged action of hydroxyl ions at a temperature of 10° and p_H only 9, or whether other factors, possibly a tendency toward reversal of the destructive process upon reacidification, are involved.

[77] **Chick and Rhodes,** Lancet, 1918, ii. 774.

[78] **Givens and McClugage,** J. Biol. Chem. 42, 491, 1920.

conditions, in 10 g. daily rations in addition to a diet of soy bean flour, milk, yeast, paper pulp, calcium lactate, and sodium chloride, which had been shown to produce scurvy in two to three weeks, resulting in death in the course of another week unless curative measures were adopted. When the raw potatoes were cooked they were chopped very finely, boiling water was poured over them, and they were kept boiling for 15 minutes. This treatment caused no appreciable reduction in the antiscorbutic potency of the potato. If the cooking was continued for an hour, however, the resulting product failed to cure or to delay the onset of scurvy.

According to **Holst** and **Frölich**[79] the juice of lemons and raspberries retain their antiscorbutic properties after heating to 100° C. for an hour, and **Delf**[80] found less reduction in *C* in the case of orange juice subjected to temperatures up to 130° C. than with either cabbage or swede juice, this greater stability being maintained even when the juice was nearly neutralized before heating. **Bassett-Smith**[81] reports that boiling lemon juice for five minutes does not appreciably diminish the antiscorbutic property, but that heating to 58° C. for three-quarters of an hour caused marked deterioration. **Davey**[82] states that orange and lemon juice lost but little in activity when preserved with the rind oil for two years at room temperature, but became inactive on keeping at 37°. **Delf** suggests that the unexpected stability of swede and orange juice at temperatures above 100° C., when the heating is conducted in the absence of air, may perhaps indicate that destruction of the antiscorbutic factor is an oxidation process.[83] This observation naturally has an important bearing on the canning and preserving of fruits and vegetables.[84]

An observation made by **Rossi**[85] is of interest in this connection. **Rossi** found that when young guinea pigs were fed a ration of oats and fresh hay which had been sterilized in an autoclave at a temperature of approximately 126° C. in open jars, they died of scurvy in a little more than 20 days. When animals of the same age were fed a ration containing the same amount of oats and fresh hay which had been sterilized in an autoclave at the same temperature but in hermetically sealed bottles, they remained in normal health after

[79] **Holst** and **Frölich**, Ztschr. Hyg. 71, 1, 1912.
[80] **Delf**, Bioch. J. 14, 211.
[81] **Bassett-Smith**, Lanc. 1920, ii, 997.
[82] **Davey**, Biochem. J. 15, 83, 1921.
[83] See also **Anderson, Dutcher, Eckles** and **Wilbur**, Sci. 53, 446, 1921.
[84] See also **Bidault**, Rev. Sci. 59, 13, 1921.
[85] **Rossi**, Arch. fisiol. 16, 125, 1918; Physiol. Abs. 4, 335, 1919.

more than two months on this diet. He concludes that foodstuffs can be sterilized at high temperatures without loss of their nutritive properties provided the proper procedure be followed.

Harden and **Robison**[86] found that about half of the antiscorbutic vitamin was destroyed in dried orange juice kept at 29° C. for 14 months. Boiling orange juice under a reflux condenser for one-half hour did not affect the antiscorbutic vitamin according to **Dutcher, Harshaw** and **Hall.**[87] On the other hand they found that this vitamin is partially destroyed by hydrogen peroxide, especially on warming.

Milk is comparatively poor in antiscorbutic power in the raw state, and the small amount present may be still further reduced by unfavorable treatment. Long before the vitamin hypothesis had been formulated, **Neumann** noted that frequent occurrence of infantile scurvy seemed to be associated with the use of milk which had been pasteurized at 90° to 95° C. and then heated again at or near 100° C. for 10 to 15 minutes before using.[88]

Frölich[89] found that raw milk had more protective power than heated milk as an antiscorbutic for guinea pigs. Autoclaved milk, i.e. milk which has been heated to 115° to 120° C. in an autoclave for one to two hours, has frequently been used as a constituent of a scorbutic diet. **Hart, Steenbock** and **Smith**[90] observed that an average consumption of 79 cc. per day of milk which had been heated to 120° C. for 10 minutes was insufficient to protect guinea pigs against scurvy, although this amount of raw milk had been found adequate. Commercial condensed milk (two brands investigated) was also lacking in *C* as compared with fresh raw milk. A commercial milk powder was found to be almost if not quite devoid of *C*, but the degree of heat to which this had been subjected was unknown. **Hess** and **Unger**[91] found that 80 cc. of fresh milk daily or its equivalent in milk dried by the **Just-Hatmaker** process (exposure to a temperature of 116° C. for a few seconds) was sufficient to cure guinea pig scurvy, while **Barnes** and **Hume**[92] found 100° to 150° cc. of raw milk necessary and got no protection with the **Hatmaker** product. They ascribe this discrepancy to the fact that **Hess** and **Unger** gave dried hay *ad lib.* in the basal ration, while **Barnes** and **Hume** used oats and bran only. Hay is probably not entirely with-

[92] **Barnes and Hume,** Bioch. J. 13, 306, 1919.
[86] **Harden and Robison,** Biochem. J. 15, 521, 1921.
[87] **Dutcher, Harshaw and Hall,** J. Biol. Chem. 47, 483, 1921.
[88] **Neumann,** Deutsche med. Wohnschr. 1902, 247.
[89] **Frölich,** Z. f. Hyg. u. Infeckt. 72, 155, 1912.
[90] **Hart, Steenbock and Smith,** J. Biol. Chem. 38, 305, 1919.
[91] **Hess and Unger,** J. Biol. Chem. 38, 293, 1919.

out *C*. The addition of scalded milk which had been heated until it frothed up at the boil and then allowed to cool in air, amounting on the whole to exposure to a temperature of between 70° and 100° C. for about five and one-half minutes, to the diet of a scorbutic monkey brought about a rapid and complete cure. The amount fed was such that about 25 per cent of the antiscorbutic factor originally present might have been destroyed without being detected, but from the rapidity of the cure it was inferred that very little destruction had occurred and such scalding is suggested as a means of sterilization involving comparatively little deterioration of the antiscorbutic power [93]

Much evidence has been accumulated with regard to the effect of drying at moderate temperatures on the *C* content of foodstuffs. **Furst** [94] noted that germinated corn lost much of its antiscorbutic power on keeping. **Holst** and **Frölich** [95] made a careful study of the effect of drying and storing on the antiscorbutic potency of potatoes, carrots, dandelion leaves and cabbage, dried in an incubator for some days at 37° C., or in vacuo at 30° C. After drying, the material was stored for six months or a year at summer temperatures, mostly in closed vessels, but in one case in open vessels in an incubator. When 30 g. per day of these vegetables in the fresh condition were fed to guinea pigs in addition to a dry grain and water diet, this amount was found sufficient to maintain the animals in health and prevent scurvy. In the case of the dried cabbage the material was soaked in water for half an hour before being fed to the animals. Any water which remained unabsorbed was fed to the animals separately. Six animals receiving cabbage dried and stored in the laboratory for one year, died with symptoms of acute scurvy in 21 to 28 days, the cabbage affording no protection whatever. Another eight animals receiving cabbage dried and stored for six months died with actue scurvy in 21 to 37 days. These also received no significant protection from the disease. With the cabbage which was stored in open vessels in an incubator for six months after drying at 37° C., a certain amount of protection was afforded but three animals ultimately died of acute scurvy, in 51 to 71 days. Better results were obtained when the cabbage had been stored only six weeks. On this, two animals died of scurvy after 66 days, and two lived for 98 days, one with no apparent symptoms and the other showing evidence of

[93] See also Ch. XV.

[94] **Furst**, Nord. Med. Arch. Abt. II, 1910, Supp. 349.

[95] **Holst and Frölich**, Ztsch. Hyg. 72, 1; 75, 334.

scorbutic lesions on histological examination of the bony tissues. In one case guinea pigs received protection for over eighty days on a ration of dried cabbage equivalent to 30 g. of the fresh vegetable; the cabbage having been dried at 37° C. and kept at that temperature in a desiccator over sulphuric acid. No increase in weight took place during the experiment. Their experiments indicate that the time of storage has a marked effect upon the antiscorbutic value of dried cabbage, and that the value is greater when the material is kept in a very dry atmosphere and prevented from taking up moisture from the air, but even in this case the value is very small in comparison with fresh material. The onset of scurvy in guinea pigs was prevented with as small an amount of fresh cabbage as 1 to 2.5 g. daily, and the most successful of the experiments with dried cabbage are far from comparable with this. Experiments with other dried vegetables showed similar deterioration on drying and storing, this being greatest with dandelion leaves and carrots, and least with potatoes.

Givens and **Cohen**[96] observed that cabbage, dried in a blast of air at 40° to 52° C., retained some of its antiscorbutic value in that it would considerably delay the onset of scorbutic symptoms, thereby prolonging life. Further, it could be employed as a curative agent if the signs of scurvy were recognized early enough and the animal would consume one gram daily. Cabbage which had been heated in an oven for two hours at 75° to 80° C., then dried at 65° to 70° C. for several days lost its antiscorbutic power. Cabbage cooked for 30 minutes, then subjected to drying for two days at 65° to 70° C., exhibited no antiscorbutic potency. **Delf** and **Skelton**[97] found that the loss of antiscorbutic properties was more than 93 per cent when cabbage was dried at a low temperature and subsequently stored for two to three weeks at laboratory temperature. Storage for five to six weeks at laboratory temperature induced a further loss of antiscorbutic properties and after storage for three months nearly all (96 to 97 per cent) of the protective value of the fresh material was lost. The residual amount was, however, distinctly greater if the cabbage was plunged into boiling water before drying. They conclude that the preservation of vegetables by drying is an unprofitable process having regard to labor and fuel involved, to the low nutritive and antiscorbutic value of the product, and to the subsequent deterioration on storage. In view of the present advocacy by others of dehydration as a useful means of preserving vegetables, this conclusion is of especial interest.

[96] **Givens and Cohen**, J. Biol. Chem. 36, 127, 1918.
[97] **Delf and Skelton**, Bioch. J. 12, 448, 1918.

Falk and **McGuire** recommend the vacuum process of drying or dehydrating foods as a means of preserving the vitamin content. The absence of oxygen during drying favors the retention of the full vitamin potency. Banana flour is advantageously prepared in this manner.[98]

Givens and **McClugage** confirmed the findings of **Holst** and **Frölich** with regard to the destructive effect of drying on the antiscorbutic factor in potatoes, the amount of destruction varying greatly with the temperature and duration of drying. Fifty-five to 60° C. is apparently the optimum temperature, 2.5 g. per day of potato dried at this temperature affording some protection. The best results were obtained with potatoes which had been baked for a short time at a high temperature and then dried. Givens and McClugage[99] remark that the influence of heat upon the antiscorbutic vitamin appears to be related not only to the degree of temperature but to the duration of the treatment, the reaction, the enzymes present, and the manner of heating. They consider a temperature of 35° to 40° C. for six to eight hours seems to be more destructive than 55° to 60° C. for two to three hours. This conclusion is based on the results obtained with potatoes dried at these temperatures and fed uncooked and cooked for 15 minutes at 100° C. With 2.5 grams of potatoes dried at 35° to 40° C. and fed without further treatment, death from scurvy was slightly delayed; with 5 grams life was certainly prolonged. One out of four animals receiving daily 2.5 grams of potatoes dried at 55° to 60° C. showed signs of scurvy at death; also one out of three animals on potatoes dried at 75° to 80° C. showed signs of scurvy at death. If these dried products are further heated in water at a temperature of 100° C. for 15 minutes and then fed to guinea pigs in amounts equivalent to 2.5 grams of dried material no protection was observed with the 35–40° C. and 75–80° C. products while with the 55–60° C. material some favorable effect was noticed.

"When potatoes were baked for a short time at a high temperature and then dried, a product was obtained which afforded protection against scurvy. Thus it appears possible that the factors involved in the destruction of the antiscorbutic vitamin are not only the degree of heat and the duration of the heating but also the enzyme content and the reaction of the food being dried. Until further light is thrown upon the problem it seems plausible to assume that at any tempera-

[98] Am. Food Jour. Oct. 1921.

[99] **Givens** and **McClugage**, J. Biol. Chem. 42, 497, 1920.

ture below 80° C. the enzymes are functionating. For example, all potatoes dehydrated below this temperature darkened during the desiccation. Furthermore, samples of these same products tested nine months after drying showed that they still contained oxidases. The temperature of 35° to 40° C. is probably the optimum one for the enzyme action. The duration of drying at this temperature, being very long, allows several hours for continuous activity. In the case of drying at 55° to 60° C. and 75° to 80° C. there is undoubtedly some enzyme action, but the time is reduced. The difference in the antiscorbutic value between the products dried at 55° to 60° C. and at 75° to 80° C. and then cooked, may be accounted for on the basis that at the higher temperature there is more destruction due to the duration of this greater heating and to enzyme action. In the case of baking and drying the enzymes are destroyed in a very short time; also the product is subjected to the high temperature for a short period and thus high heat and reaction have little time to bring about a joint effect.

Drying at 35° to 40° C. for 32 to 52 hours appears to reduce the *C* content of tomatoes somewhat, the loss being greater if the dried tomatoes are cooked before feeding.[100]

Bassett-Smith[101] kept a preparation of antiscorbutic material from lemon juice for three months at temperatures of 15°, 30° and 37° C. without appreciable loss of antiscorbutic power. The temperature of 37° was least satisfactory, the vitamin being slightly affected in this case.[101a]

Harden and **Robison**[102] report that the antiscorbutic property of orange juice is not appreciably destroyed by evaporation to dryness under suitable conditions. The dry residue retains considerable activity after storage for two years in a dry atmosphere at ordinary temperature, an observation which is confirmed by **Givens** and

[100] **Givens** and **McClugage**, Proc. Soc. Biol. Chem.; J. Biol. Chem. 41, xxiv, 1920.

[101] **Bassett-Smith**, Lancet 1920, 1102.

[101a] The preservation of citrus fruit juices from the standpoint of antiscorbutic potency has been studied by **Davey** (Biochem. J. 15, 83, 1921). Potassium metabisulphite is satisfactory at 0° but it is uncertain at room temperature and is ineffective at 37° C. Preservation with the rind oil, in the case of lemon and orange is satisfactory at temperatures up to room temperature. Even after two years the potency is very little diminished. Citrus fruit in cold storage therefore should maintain a high degree of antiscorbutic value for an indefinite period. At 37° C. deterioration is rapid.

[102] **Harden** and **Robison**, Biochem. J. 1920, 14, 171–177.

Macy.[103] **Hess and Unger,**[104] on the other hand, found that orange juice which had been kept in a refrigerator for some time had lost some of its nutritive value as indicated by the improvement in weight and general condition observed in the case of a guinea pig when the refrigerated juice was replaced by only half as much fresh orange juice in its dietary.

Givens and **Macy**[105] have investigated the antiscorbutic properties of fruit juices dried by a commercial process. Lemon juice, acid and neutralized, tomato juice, grapefruit, and orange juice all contained a significant amount of the antiscorbutic vitamin; grape and raspberry juice were apparently devoid of it. The preparations used were for the most part 14 to 20 months old.[105a]

Certain observations have suggested that enzyme action may be an important factor in the gradual destruction of the antiscorbutic vitamin and to test this suggestion, **Smith** and **Medes**[106] heated orange juice, both in the presence and absence of an enzyme, at temperatures of 38°, 55° and 76° C. If enzyme activity causes destruction of the vitamin, presumably disappearance would be most rapid at 55° C., the temperature at which the activity of the enzyme is greatest. The enzyme employed was invertase, since it is a normal constituent of orange juice. This enzyme did not contribute to the destruction of the antiscorbutic vitamin when heated with the vitamin for four hours at 38°, 55° and 76° C. This does not preclude the possibility that other enzymes may have such an effect. Incidentally, Smith and Medes note that heating for four hours at a temperature of 76° C., either in the presence of invertase or in its absence, causes a more rapid destruction of the vitamin than heating at 55° C. Heating for four hours at 38° C., does not cause an appreciably greater loss of antiscorbutic value than keeping at room temperature.

Beef, which is poor in *C,* loses its antiscorbutic power completely when it has been dried in vacuo at 65° C., or lower, for 12 hours and then air-dried for several days.[107]

[103] **Givens** and **Macy,** Proc. Am. Soc. Biol. Chem.; J. Biol. Chem. 46, xi, 1921.

[104] **Hess** and **Unger,** J. Biol. Chem. 35, 487, 1918.

[105] **Givens** and **Macy,** *loc. cit.*

[105a] The effect of drying vegetables in the sun has been investigated by **Shorten** and **Ray** (Biochem. J. 15, 274, 1921). Dried tomatoes, potatoes and cabbage retain much of their antiscorbutic value. This is not true, however, of carrots, spinach or turnips. Dried cabbage, tomatoes, carrots, spinach and potatoes showed antineuritic properties.

[106] J. Biol. Chem. 48, 323, 1921.

[107] **Givens** and **McClugage,** Sci. 51, 273.

That very low temperatures may be deleterious as well as higher temperatures is indicated by **Wiltshire's** experience in Siberia, where scurvy broke out among the troops, although each man received a ration of frozen meat daily.[108]

[108] **Wiltshire**, Lancet, 1918, ii, 811.

A summary of the destructive effects of oxidation and catalysis on vitamins is given by **Hess** (J. Ind. Eng. Chem. 1921, 1115).

CHAPTER VI

CHEMICAL STRUCTURE AND PROPERTIES OF THE VITAMINS

Nothing is yet known concerning the chemical structure, and very little concerning the properties of the fat-soluble vitamin. Most of the observations that have been made on it have to do with its solubility.[1] **McCollum** and **Davis**[1a] found that ether extracted this substance from egg-yolk and butter-fat, but **McCollum** and **Simmonds**[2] failed to extract it from plants by such solvents as ether, chloroform, benzene, and acetone, and therefore concluded that it was not found in any fats or oils of plant origin. **McCollum, Simmonds** and **Pitz**[3] state that the residue from corn meal after extraction with ether is more effective in causing a slow but long-maintained upward trend of the curve of growth than is corn oil. Ether extraction of plant tissue therefore does not necessarily remove the vitamin. They suggest that the fat-soluble *A* is in chemical union in the plant tissues, in some complex which is not soluble in fat or in ether. In digestion and absorption it is set free, and being readily soluble in fats thereafter accompanies the fats in the animal body. Owing to the large content of waxes, etc., extracted from plant leaves they have not been very successful in feeding ether extracts from these sources. **Osborne** and **Mendel**,[4] however, obtained a very effective extract from spinach and clover with ether. **Steenbock** and **Boutwell**[5] found that there was great variation in different substances as to the ease with which the fat-soluble vitamin was yielded to solvents. While ether in general was rather a poor solvent, it was nevertheless possible to obtain a fairly satisfactory ether extract of alfalfa but not of carrots. Chloroform and carbon disulphide remove some of the fat-soluble vitamin as found in carrots, while alcohol and benzene remove considerable amounts of it. The results were not entirely satisfactory, however, as the extracted carrot residues were almost as effective as the orig-

[1] **LaMer** considers vitamins from the standpoint of physical chemistry. (J. Ind. Eng. Chem. 1921, 1108).

[1a] **McCollum and Davis**, J. Biol. Chem. 1913, 15, 167.

[2] **McCollum and Simmonds**, *Ibid*, 32, 33, 1917.

[3] **McCollum, Simmonds and Pitz**, Am. J. Physiol. 41, 361, 1916.

[4] **Osborne and Mendel**, Proc. Soc. Exp. Bi. Med. 16, 98, 1918-19.

[5] **Steenbock and Boutwell**, J. Biol. Chem. 42, 131, 1920.

inal carrots when fed in small amounts as the sole source of *A*. Evidently while carrots contain considerable amounts of *A*, for some reason or other no satisfactory quantities of the vitamin could be accumulated from them. These investigators suggest that this may be on account of the presence of the colloidal pectins and hemicelluloses or possibly on account of the occurrence of the vitamin in molecular combinations. Fat solvents applied to alfalfa gave far more satisfactory results. Alcohol was found to be the most satisfactory solvent, but benzene was also effective. Water proved very poor. With corn, ether failed to extract the vitamin, but alcohol,[6] cold or hot, removed it quantitatively. Although dried tomatoes contain considerable amounts of the fat-soluble vitamin, the ether extract had no growth-promoting power.[7]

Drummond[8] found that *A* is not extracted from oils by water or dilute acid but that it may be removed in small quantity by cold extraction with alcohol. He could find no evidence which suggests that *A* is not a single substance, but was unable to identify it with any of the recognized components of fats, such as glycerol, saturated or unsaturated fatty acids, cholesterol, lecithin, phosphatides, or lipochromes. He suggests that it may be a labile substance of ill defined construction.

According to **Drummond**,[9] saponification in non-aqueous media of oils containing the accessory factor results in the disappearance of the growth-stimulating properties, although an earlier experiment[10] had shown that a high grade whale oil which had been treated with soda to remove the free fatty acids and subsequently clarified with fuller's earth was still rich in *A*.

Steenbock and **Boutwell**[11] found that *A* as extracted from alfalfa with alcohol resists saponification with alcoholic potassium hydroxide in the cold. From such solution on dilution with water it can be extracted with ether. On fractionation by differential solubility there can be obtained a portion soluble in petroleum ether, containing *A* in large amounts, along with carotin, and a dilute alcohol-soluble portion which contains xanthophyll, but little or no *A*.[12] This is in accordance with the observation of **McCollum** and **Davis**[13] that

[6] See also **McCollum, Simmonds** and **Pitz**, J. Biol. Chem. 28, 153, 1916.

[7] **Osborne** and **Mendel**, J. Biol. Chem. 41, 549, 1920.

[8] **Drummond**, Bioch. J. 13, 81, 1919.

[9] **Drummond**, Bioch. J. 13, 81, 1919.

[10] **Drummond**, J. Physiol. 52, 344, 1918.

[11] **Steenbock** and **Boutwell**, J. Biol. Chem. 42, 131, 1920.

[12] **Steenbock** and **Boutwell**, l. c.

[13] **McCollum** and **Davis**, J. Biol. Chem. 19, 245, 1914.

the property of inducing growth in rats which have grown as far as possible on a fat-free ration can be conferred on olive oil by shaking the latter with a solution of the soaps prepared by completely saponifying butter-fat in a non-aqueous medium with potassium hydroxide. According to **McCollum** and **Davis**, the results obtained leave no room for doubt as to the stimulating effect of this oil as compared with the untreated oil.

More recently, **Steenbock, Sell** and **Buell** [13a] have investigated the stability of *A* as found in animal fats, and state that no appreciable destruction of the vitamin could be observed after boiling the fat for ½ hour with 20 per cent alcoholic KOH.

In recent years a large amount of vegetable oil has been treated by the hydrogenation process to form solid fats known as hardened oils. The process as usually carried out involves the treatment of the oil with hydrogen gas in the presence of a nickel catalyzer. The temperatures generally employed range from 150° to 190° C. The time of treatment with hydrogen for the hardening operation ranges from one to eight hours.[14] In view of the effect of high temperatures on vitamins it is to be expected that their activity would decrease during the hardening process. This is apparently the case as is indicated by the investigations of **Halliburton, Paton, Drummond** and others [15] who found fat-soluble vitamins to be absent in oils treated by the hydrogenation process.

However, recent investigations pointing to the stability of vitamins toward heat, in the absence of air or oxidizing agents, suggest other causes than heat alone. It is not unlikely that the catalytic action of the nickel catalyzer may be exerted on the vitamins thereby changing their character.[15a] **Fahrion** [15b] refers to the therapeutic value of cod liver oil due to vitamins. Oil extracted by cold pressing contains the vitamins in a potent state. He notes, however, that the oil obtained by solvent-extraction does not contain these food factors. As has been stated, refining by alkali destroys vitamins. Fuller's earth probably absorbs vitamins during the bleaching operation.

It follows that lard substitutes made from refined vegetable oils which have been thickened to the consistency of lard by the addition of 15 or 20 per cent of hydrogenated oil, scarcely can be expected to contain vitamins. Various suggestions have been made for adding to

[13a] **Steenbock, Sell and Buell,** J. Biol. Chem. 47, 89, 1921.

[14] See **Ellis,** Hydrogenation of Oils, New York, 1919.

[15] **Halliburton, Paton, Drummond** and others, J. Physiol. 1919, 52, 325.

[15a] See **Drummond,** J. S. C. I., 1921, 81 T.

[15b] Chem. Umshau, 1920, 97 and 110.

oleomargarine and compound lard suitable extracts of vitamin *A*.[15c]

The result of the investigations of Halliburton, Drummond and others on the absence of vitamins in processed vegetable oils have led to much controversy over the relative merits of animal and vegetable fats, of milk and butter, oleomargarine, vegetable oil, compound lard etc. **Richardson**[15d] takes issue with the champions of the virtues of milk and butter. With a varied diet, he believes that it makes no difference whether oleomargarine or butter, in fact any dairy product, is consumed.

If rats, the experimental animals most used in the past for dietetic experiments, are fed a mixed ration of moderate variety, consisting of the food commonly used on the ordinary table, meat, vegetables, grains and fruits, but without milk or dairy products, they get along very well indeed, without developing dietary or deficiency diseases, showing normal growth and health, except for an occasional ailment or infection to which such laboratory animals are always subject. Such rats reproduce unto the nth generation in spite of the absence of their per diem of milk. When to such a diet either oleomargarine or butter is added no noteworthy change results which, Richardson notes, is as might have been foreseen and expected. Rabbits and guinea pigs grow to maturity, flourish and reproduce on green things as do the large herbivorous animals, while the carnivorous kinds eschewing vegetable products subsist entirely on meat. These would commonly be considered to be most unbalanced diets, but the addition of dairy products does not change the course of nature. Large quantities, however, might and sometimes do cause serious disorders, since milk and dairy products are very different in some of their properties from the natural foods of certain animals.

Osborne and **Mendel**[16] claim that the centrifuged butter-fat from which they obtained the growth-promoting factor contained neither nitrogen nor phosphorus. This was doubted by **McCollum** and **Davis**,[17] but confirmed by the analyses of **Folin** and of **Funk** and **Macallum**,[18] who found only 0.0003 per cent of nitrogen, and of **Osborne** and **Wakeman**,[19] who estimate the phosphorus content of the butter oil in which the growth-promoting principle is concentrated at 0.0006 per cent. In view of these figures it seems impossible that either phosphorus or nitrogen should form a constituent part of the fat-soluble vitamin.

[15c] Experiments made in the laboratory of the senior author by adding an extract of alfalfa to oleomargarine (nut butter) were not very promising owing to the foreign flavor imparted to the product.

[15d] **Richardson**, Am. Food Jour., July, 1921.

[16] **Osborne** and **Mendel**, J. Biol. Chem. 20, 382, 1915.

[17] **McCollum** and **Davis**, J. Biol. Chem. 19, 250, 1914.

[18] **Funk** and **Macallum**, Zeitsch. f. physiol. Chem. 92, 13, 1914.

[19] **Osborne** and **Wakeman**, J. Biol. Chem. 21, 91, 1915.

Normal growth and reproduction has been observed in albino rats, a species of animal in which there is a complete absence of carotinoid pigments, with ewe milk-fat and carotinoid-free egg yolk as the sole sources of fat-soluble vitamin. The ewe milk-fat contained only 0.00014% of carotin and the ration showing the best results contained only 0.0000126% of carotin. The basal ration used was entirely devoid of carotinoids. A quantitative comparison of the carotin content and vitamin efficiency of various rations shows no relationship. Carotinoid pigment is not necessary for the normal growth and reproduction of albino rats and apparently the fat-soluble vitamin and carotinoid pigment are not identical.[19a]

The antineuritic or water-soluble *B* vitamin is, as the name implies, generally regarded as soluble in water [20] but **Cooper** [21] found that the antineuritic content of meat is not extracted by water although it is readily soluble in alcohol, after which it becomes soluble in water. From lentils *B* can be extracted directly by water.[22] **Suguira** [23] prepared a sparingly soluble compound from yeast which had antineuritic properties. It would seem that the antineuritic, like the fat-soluble vitamin, may vary in solubility according to the condition in which it is found. Many observers [24] have reported its solubility in ethyl alcohol, and this is commonly used as an extractive. **Osborne** and **Mendel** [25] have pointed out that the term *alcohol* is sometimes rather carelessly used, without specifying whether absolute or dilute alcohol is referred to. They state that in their experience absolute alcohol is by no means an adequate solvent for the antineuritic vitamin and that the solubility in dilute alcohol depends on the amount of water present. This is confirmed by **Drummond** [26] and by **Osborne, Wakeman** and **Ferry.**[27]

Osborne and **Wakeman** [28] added alcohol gradually to a 0.01 per cent N. acetic acid extract of yeast vitamin and tested the different fractions for growth-promoting qualities. That obtained from 52 per

[19a] **Palmer** and **Kennedy,** J. Biol. Chem. 46, 559, 1921.

[20] **Chamberlain** and **Vedder,** Phil. J. Sci., B6, 395, 1911; **McCollum** and **Davis,** J. Biol. Chem. 23, 181, 1915.

[21] **Cooper,** J. Hyg. 12, 436.

[22] **Cooper,** l. c.

[23] **Suguira,** J. Biol. Chem. 36, 191.

[24] **Stepp,** Tschr. Zt. f. Biol. 57, 135; **Eijkman,** Arch. f. path Anat, 148, 523, 149, 197; **Fraser** and **Stanton,** Lancet, March 12, 1910, 733; **Chamberlain** and **Vedder,** Phil. J. Sci. 6B, 395; **Tervvchi,** Y., Saiking akuashi, Tokio, 1910, No. 179; Zeits. Bioch. Biophys. 11, 719; **Funk,** Trans. Soc. Trop. Med. 5, 86.

[25] **Osborne** and **Mendel,** J. Biol. Chem. 31, 149.

[26] **Drummond,** Bioch. J. 11, 255, 1917.

[27] **Osborne, Wakeman** and **Ferry,** J. Biol. Chem. 39, 35, 1919.

[28] **Osborne** and **Wakeman,** J. Biol. Ch. 40, 383, 1919.

cent alcohol (by weight) was almost inert, while that soluble in 52 per cent, but precipitated by 79 per cent contained most of the active substance. That precipitated from 90 per cent alcohol was almost as active per unit as that from 79 per cent, but the amount obtained was small, only 10 per cent of the total extract, while the second fraction contained 37 per cent of the total. The portion soluble in 90 per cent alcohol also contained the vitamin, but not in so great a concentration as the preceding.

On the other hand, **Funk**[29] and **Fraser** and **Stanton**[30] have successfully used absolute alcohol to extract the antineuritic substance from rice polishings and **Cooper**[31] has made use of it for extraction of various foodstuffs.

Vedder and **Williams**[32] found the vitamin from rice only slightly soluble in cold 95 per cent alcohol. **McCollum** and **Simmonds**[33] state that it is very difficult to extract it completely from cooked beans. They believe that the failure of alcoholic extracts of cooked beans to induce growth is due to the physical changes in the bean during cooking whereby the vitamin is protected from contact with the solvent, not because of destruction of the active substance. Even with raw beans extraction with hot alcohol in a Soxhlet was not complete in 18 hours. **Eddy**[34] found it possible to extract the vitamin from sheep's pancreas with cold 95 per cent alcohol containing 0.8 per cent HCl. Methyl alcohol dissolves the vitamin from yeast[35] and from some vegetables.[36]

In general the antineuritic vitamin appears to be insoluble in ether,[37] although **Cooper**[38] finds that it can be extracted from egg-yolk, and to a lesser degree from meat, by this solvent.

It is soluble to some degree in acetone, as noted by **Stepp**[39] and confirmed by **McCollum** and **Davis**,[40] who state, however, that it is a poorer solvent than water or alcohol. **McCollum** and **Simmonds**[41]

[29] **Funk**, J. Physiol. 43, 395, 1911–12.
[30] **Fraser** and **Stanton**, Lancet, 1910, ii, 1755.
[31] **Cooper**, J. Hyg. 12, 436, 1913.
[32] **Vedder** and **Williams**, Phil. J. Sci. 8B, 175.
[33] **McCollum** and **Simmonds**, J. Biol. Chem. 33, 55, 1918.
[34] **Eddy**, J. Biol. Chem. 27, 113, 1916.
[35] **Myers** and **Voegtlin**, J. Biol. Chem. 42, 199, 1920.
[36] **Brill** and **Alencastre**, Phil. J. Sci. 12A, 127, 1917.
[37] **Stepp**, Zeitschr. f. Biol. 57, 135; **Chamberlain**, **Vedder** and **Williams**, Phil. J. Sci. 7B, 39, 1912; **Cooper**, Bioch. J. 7, 268, 1913; 8, 347, 1914.
[38] **Cooper**, J. Hyg. 12, 436.
[39] **Stepp**, Zeitschr. f. Biol. 62, 405, 1913.
[40] **McCollum** and **Davis**, J. Biol. Chem. 23, 192, 1915.
[41] **McCollum** and **Simmonds**, J. Biol. Chem. 33, 55, 1918.

found that *B* is not extracted directly from beans, wheat germ, or pig kidney by ether, benzene, or acetone, but is readily extracted in great part by alcohol. After being removed by alcohol it is soluble in benzene, but very slightly in acetone.

McCollum and **Kennedy**[42] report that the vitamin from fat-free wheat embryo can be extracted by acetone, benzene, and ethyl acetate, but **McCollum** and **Simmonds**[43] noted that the scheme of applying successively to raw navy beans the solvents ether, benzene, and 95 per cent alcohol, in order named, and of feeding the extract and residue in separate experiments, showed that benzene does not remove *B* from ether-extracted beans while hot alcohol does. When material which was dissolved from beans by hot alcohol was deposited on dextrin and the latter then extracted with hot benzene, the benzene-soluble material thus obtained was very effective in inducing growth and in causing prompt recovery of polyneuritic rats. A benzene extract prepared directly from ether-extracted beans does not contain an appreciable amount of *B*. Benzene therefore does not remove *B* from beans directly, but once this substance is extracted by alcohol it is soluble in benzene.

Steenbock[44] states that he was able to cure polyneuritis in pigeons by means of intraperitoneal injections of a water-acetone soluble substance from egg-yolk.

Cooper[45] prepared a curative substance from horse-flesh which was moderately soluble in water, and insoluble in alcohol, benzene, chloroform, ether, and ethyl acetate, and states that the brain contains an antineuritic substance which can be extracted with alcohol and precipitated by a mixture of acetone and ether.

Myers and **Voegtlin**[46] found that olive oil and oleic acid remove the vitamin from autolyzed yeast filtrate, thus showing that in the form of the crude extract it is fat-soluble as well as water-soluble.

Water-soluble *B* is not colloidal, since it can be dialyzed through parchment[47] and collodion[48] membranes.

It is readily absorbed by charcoal,[49] kaolin,[50] mastic,[51] fuller's

[42] **McCollum and Kennedy,** J. Biol. Ch. **24,** 491.

[43] **McCollum and Simmonds,** J. Biol. Chem. **33,** 55, 1918.

[44] **Steenbock,** Proc. Am. Physiol. Soc. Am. J. Physiol. **42,** 610.

[45] **Cooper,** Bioch. J. **7,** 268, 1913; **8,** 347, 1914.

[46] **Myers and Voegtlin,** J. Biol. Chem. 42, 199, 1920.

[47] **Eijkman,** l. c.; **Chamberlain and Vedder,** Phil. J. Sci. 6B, 395, 1911; **Drummond,** J. C. Bioch. J. **11,** 255, 1917.

[48] **Suguira,** J. Biol. Chem. 1918, **36,** 191.

[49] **Chamberlain and Vedder,** Phil. J. Sci. **6B,** 251, 395; **Voegtlin,** J. Wash. Acad. Sci. Oct. 4, 1916.

[50] **Voegtlin,** J. Wash. Acad. Sci. Oct. 4, 1916.

[51] **Voegtlin,** J. Wash. Acad. Sci. Oct. 4. 1916.

earth,[52] **Lloyd's** reagent (colloidal hydrous aluminum silicate,[53] colloidal ferric hydroxide,[54] and, according to **Drummond**, by flocculent precipitates of all kinds, a property which doubtless accounts for some, at least, of the losses observed in attempting to purify this factor, although **Funk**[55] regards this loss as unimportant.

Emmett and **McKim**[56] state that the yeast vitamin is not absorbed by kieselguhr or infusorial earths, but **Brill**[57] found that the curative principle from rice polishings is extracted by infusorial earth, although not by as small a proportion of the earth as was used of the Lloyd reagent by **Seidell**. **Brill** considers this partial extraction to be due to the character of the extract. There appears to be a loss of antineuritic power in the extract as it ages. He regards the method inapplicable, because of the quantity of earth needed completely to extract the total vitamin content.

The water-soluble vitamin appears to be stable towards acids.[58] Alcohol saturated with hydrochloric acid is frequently used to extract it from the food substances in which it is contained.[59] **McCollum** and **Simmonds**[60] found some indication that it may be slowly inactivated by this acid, but the evidence is not conclusive, and the action is certainly inconsiderable. Similarly with nitrous acid,[61] the vitamin may be destroyed to some slight extent, but very slowly if at all, and the same may perhaps be true of sulphuric acid.[62]

On the other hand, after hydrolysis with mineral acids, extracts of rice polishings have been found to be more efficacious as an antineuritic factor than before such treatment,[63] a result which may be

[52] **Seidell**, U. S. Patent 1, 173, 317; **Emmett** and **McKim**, J. Biol. Chem. **32**, 403, 1917; Public Health Reports Feb. 18, 1916, p. 364. Reprint No. 325; **Harden** and **Zilva**, Bioch. J. **12**, 93, 1918.

[53] **Seidell**, U. S. Patent 1, 173, 317; **Emmett** and **McKim**, J. Biol. Chem. **32**, 409, 1917; Public Health Reports Feb. 18, 1916, p. 364. Reprint No. 325; **Eddy** and **Roper**, Proc. Soc. Exp. Biol. Med. **14**, 52, 1916.

[54] **Harden** and **Zilva**, Bioch. J. **12**, 93, 1918.

[55] **Funk**, Biochem. Bull. **5**, 1, 1916.

[56] **Emmett** and **McKim**, J. Biol. Chem. **32**, 409.

[57] **Brill**, Phil. J. Sci. **12**, 199, 1917.

[58] **Funk**, l. c.; **Voegtlin**, Wash. Acad. Sci. Oct. 4, 1916; Abstr. in Nature, **98**, 373; **Steenbock**, Proc. Am. Physiol. Soc. Am. J. Physiol. **42**, 610, 1917; **Whipple**, J. Biol. Ch. **44**, 175, 1920.

[59] **Funk**, l. c.; **Eddy**, J. Biol. Chem. **27**, 113, 1916; **Brill** and **Alcastre**, Phil. J. Sci. **12A**, 127, 1917; **Myers** and **Voegtlin**, J. Biol. Chem. **42**, 199, 1920.

[60] **McCollum** and **Simmonds**, J. Biol. Chem. **33**, 55, 1918.

[61] **McCollum** and **Simmonds**, J. Biol. Chem. **33**, 55, 1918.

[62] **Drummond**, Bioch. J. **11**, 255, 1917.

[63] **Vedder** and **Williams**, Phil. J. Sci. B. 1913, **8**, 175; **Williams** and **Saleeby**, *Ibid*, 1915, **10**, 99; **Sullivan** and **Voegtlin**, Proc. Soc. Biol. Chem. 1915, J. Biol. Chem. **24**, xvii, 1915–16.

due to the liberating of the active constituent from a less active compound present in the original extract.[64]

Osborne and **Wakeman**[65] prepared an extract from yeast by running washed brewers yeast into boiling 0.01 N. acetic acid, the extract thus prepared being said to contain almost all the growth-promoting vitamin of the yeast. An attempt to precipitate the vitamin fractionally from the evaporated filtrate by means of increasing concentrations of added alcohol was, however, only partially successful.

It is said[66] to be stable to concentrated alkalis at room temperature, but the evidence regarding its sensitiveness to dilute alkalis at boiling temperature is conflicting. Boiling for an hour with 0.28 per cent sodium hydroxide solution destroyed the antineuritic content of wheat embryo almost entirely.[67] **Fraser** and **Stanton**[68] inferred from their experiments with rice polishings that 0.5 per cent sodium hydroxide solution destroyed the antineuritic substance.

Voegtlin and **Lake**[69] produced typical symptoms of polyneuritis in cats and dogs by feeding meat which had been treated with a 10 per cent sodium carbonate solution until distinctly alkaline and then heated to 120° C. for three hours. Meat which had been heated to this temperature for three hours without the addition of alkali had not completely lost its antinueritic power. Commenting on this work,[70] **Osborne** and **Mendel** say:

> **Voegtlin** and **Lake** assume that their ground beef muscle which was treated with 10 per cent sodium carbonate solution until the tissue reacted strongly alkaline to litmus and then heated at 120° under pressure for three hours, was free from *B*, but six rats whose sole food consisted of meat thus treated, lived for more than three months and gained in weight, one even living for about seven months and gaining 150 per cent in weight. **Osborne** and **Mendel**[71] found that dried lean round of beef (untreated) furnishes so little of this vitamin that animals fail almost as quickly when this is the sole source of protein as when the extracted meat residue is used. Contrasting **Voegtlin** and **Lake's** results with those obtained by **Osborne** and **Mendel** with meat which was extracted with boiling water only, it is difficult to believe that the treatment with alkali was responsible for loss of vitamin. They cannot account for the good results with rats on this diet.

[64] **Williams** and **Seidell**, J. Biol. Chem. 26, 431, 1916; **Daniels** and **McClurg**, *Ibid.* 37, 201, 1919.

[65] **Osborne** and **Wakeman**, J. Biol. Chem. 40, 383, 1919.

[66] **Steenbock**, l. c.; **Williams** and **Seidell**, J. Biol. Chem. 26, 431, 1916.

[67] **McCollum** and **Simmonds**, l. c.

[68] **Fraser** and **Stanton**, Lancet 1915, i, 1021.

[69] **Voegtlin** and **Lake**, J. Pharmacol. II, 167, 1918; Am. J. Physiol. 47, 558, 1918.

[70] **Osborne** and **Mendel**, J. Biol. Chem. 39, 42, 1919.

[71] **Osborne** and **Mendel**, J. Biol. Chem. 32, 309.

Daniels and McClurg[72] failed to observe any appreciable effect on the vitamin content of beans when boiled with dilute alkali for thirty minutes, but this was probably due to the fact that their rations contained so large an excess of the vitamin that destruction of almost 50 per cent of it might remain undetected. **Whipple,**[73] using the **Williams'** yeast test (q. v.) for the presence of vitamin, came to the conclusion that the water-soluble vitamin in cabbage is not destroyed by boiling with a 0.1 per cent sodium bicarbonate solution.

Drummond * found that boiling with five per cent sodium hydroxide for five hours tends to destroy the growth-promoting properties of yeast.

Miller,[74] using the same method, found that cooking navy beans in 0.5 per cent sodium bicarbonate solution for one hour and ten minutes caused a loss of 37.5 per cent of the vitamin.

Osborne, Wakeman and **Ferry**[75] digested brewers' yeast with ten times its weight of 0.1 N. sodium hydroxide for twenty-one and one-half hours, and then heated it on the water bath for two hours. After this treatment its growth-promoting properties, as tested on rats which had begun to lose weight on a vitamin-free diet, were found to be as pronounced as those of fresh yeast. More recently, however, **Osborne** and **Leavenworth**[75a] have reported that while the efficiency of a yeast preparation was not appreciably impaired by being dissolved in 0.1N alkali for 18 hours at 20 C. exposure to these conditions for 90 hours or heating with alkali for a short time resulted in serious deterioration. They conclude that it is allowable to use dilute alkali solutions in the concentration or isolation of *B* provided the temperature is kept low and the time of exposure to the action of the alkali is short.

Since water-soluble *B* is less stable to heat treatment in the presence of alkalis, there is ground for believing that biscuit baked with soda are not so nutritious as when the flour is used to make raised bread. The occurrence of pellagra in certain sections of the South is held by some authorities to be due to the almost exclusive use of soda biscuit instead of raised bread.[75b]

[72] **Daniels and McClurg,** J. Biol. Chem. 37, 201, 1919.
[73] **Whipple,** J. Biol. Chem. 44, 175, 1920.
* **Drummond,** Bioch. J. 11, 255, 1917.
[74] **Miller,** J. Biol. Chem. 44, 159, 1920.
[75] **Osborne, Wakeman** and **Ferry,** J. Biol. Chem. 39, 35, 1919.
[75a] **Osborne** and **Leavenworth,** J. Biol. Chem. 45, 423, 1921.
[75b] Am. Food Jour. Oct. 1921.

To explain conflicting results of different observers, **Daniels** and **McClurg**[76] suggest that destruction, where partial, may escape observation; or that antineuritic vitamin may be present in tissue in two forms, one so bound chemically that it is unaffected by treatment which is destructive to the free or unbound form. However, this hypothesis fails to account for all the discrepancies noted.

The attempts to separate the antineuritic vitamin from the organic matter with which it is associated fall into two classes, those in which the aim is to separate a chemically-pure substance which may be analyzed and identified, and those in which the object is merely to obtain the curative substance in concentrated form. The latter will be treated subsequently and here we shall concern ourselves only with the efforts to isolate a pure substance with curative power.

These have led in many cases to the separation of crystalline compounds which have been found to exert a considerable restorative effect in the treatment of polyneuritis, but usually the curative property disappears as the substance is purified, and the chemical constitution of the vitamin remains an open question. The most extensive work in this field has been done by **Funk** and by **Williams.** The materials used as a source of vitamin are usually rice polishings or yeast.

The first attempt to isolate vitamin by the fractionation of rice polishings was made by **Funk**[77] as follows: Rice polishings were extracted with cold absolute alcohol, containing two to five per cent of hydrochloric acid. The extracts were evaporated in vacuo at a low temperature, and the fatty residue melted and extracted with water. These aqueous extracts, after addition of sulphuric acid to the extent of five per cent, were precipitated with 50 per cent phosphotungstic acid solution and the precipitate decomposed with baryta in the usual manner. The solution, entirely freed from baryta and sulphuric acid, was filtered, and the filtrate neutralized with hydrochloric acid and evaporated in vacuo. The residue was extracted with alcohol and the solution freed by filtration from inorganic chlorides. The alcoholic solution was then precipitated with alcoholic mercuric chloride solution. The active substance was found to a small extent in this precipitate but the bulk was in the filtrate. From each of these fractions the vitamin could be completely thrown down

[76] **Daniels** and **McClurg**, J. Biol. Chem. 37, 201, 1919.

[77] **Cooper** and **Funk**, Lancet, 1911, ii, 1266; **Funk**, J. Physiol. 43, 395, 1911. **Schaumann**, Arch. f. Schiffs, u. Trop. Hyg. 16, 825, 1921, claims to have isolated the antineuritic substance from rice polishings before **Funk**, but did not identify it. **Funk**, J. Physiol. 45, 489, asserts that the substance obtained according to the method used by **Schaumann** could only have been allantoin.

by silver nitrate and baryta. From this fraction, which contains the histidine, pyrimidine, and nicotinic acid groups, after decomposition with hydrogen sulphide, there was isolated a very small quantity of crystalline substance, with a melting point of 233° C. This was not recrystallized, but possessed very marked curative power. The only substance isolated from 54 kilos of rice polishings were this crystalline material (which, from our present knowledge, was undoubtedly impure nicotinic acid) and a large quantity of choline. This work proved that vitamin is quantitatively precipitated by phosphotungstic acid, and by silver nitrate and baryta, partially by mercuric chloride in alcoholic solution, but not by platinic chloride or picric acid.

Shortly after this work was done there appeared a paper on this subject by **Edie, Evans, Moore, Simpson,** and **Webster,**[78] who used a slightly modified method. The source of their material was yeast. This was extracted with alcohol, and the alcohol extract evaporated, cleared with lead acetate, and immediately precipitated with silver nitrate. This precipitate, when decomposed, gave a small quantity of crystalline organic substance that yielded ash. These investigators gave the name *torulin* to the substance isolated, but did not claim to have tested its curative power.

Later, in 1912, **Funk** endeavored to isolate the vitamin from different foodstuffs,[79] e. g., yeast, rice polishings, ox-brain, milk,[80] and lime juice.[81] An extract of rice polishings was precipitated directly with silver nitrate and baryta. This process was chosen to shorten the procedure and to avoid the use of a large quantity of alkali, which was supposed to destroy the vitamin. No vitamin was detected in this case. Allantoin was found, which, when phosphotungstic acid is used, goes into the filtrate. The evaporated alcoholic extract of yeast was hydrolyzed with 10 per cent sulphuric acid solution for a short time previous to precipitation with phosphotungstic acid, and the hydrolyzate was worked up in a way similar to that for the first fractionation of the extract of rice polishings, with the difference that mercuric chlorid was not used. The silver-fraction when decomposed yielded a crystalline substance, melting at 233° C., that gave a precipitate with mercuric acetate, but not with mercuric sulphate or nitrate. No copper salt was formed in boiling water with copper oxide. The yield, 0.45 g. from 75 k. of dry yeast, was too small for

[78] **Edie, Evans, Moore, Simpson,** and **Webster,** Bioch. J. 6, 234, 1912.
[79] J. Physiol. 45, 75, 1912.
[80] Bioch. J. 7, 211.
[81] *Ibid,* 81.

recrystallization or analysis; a rather large dose of the substance (0.02 to 0.04 g.) proved, however, to be curative for beriberi pigeons. In another fractionation of yeast the alcoholic extract was simply extracted with water and not hydrolyzed; in this case the substance described above could not be isolated but some pyrimidine bases, e. g. uracil and thymin, were obtained.

In a later attempt [82] to obtain the vitamin from yeast, a crystalline product was obtained from the silver and baryta fraction, which melted at a temperature above 200° C., and which when administered to beriberi pigeons induced very quick recovery. When, however, maintenance experiments were performed with pigeons on a diet of polished rice given daily injections of the product, the birds could not be kept alive for more than a few days. The product of this first crystallization was divided into three substances, one of which was nicotinic acid, the other with the composition $C_{24}H_{19},O_9N_5$ and $C_{29}H_{23}O_9N_5$ respectively. The administration of these substances, individually or collectively, to beriberi pigeons did not show appreciable curative action.

From the vitamin fraction of rice polishings two crystalline products were obtained, one of which proved to be nicotinic acid and the other having the formula $C_{26}H_{20}O_9N_4$.

In the same year **Suzuki, Shinamura** and **Odake** [83] published a paper on the chemistry of rice polishings. Their method of isolating the active principle was as follows: The rice polishings were first extracted with petroleum ether, to remove the fat, and then with boiling alcohol. The evaporated alcoholic extracts were diluted with 3 per cent sulphuric acid solution, and precipitated with phosphotungstic acid. The precipitate, after decomposition with baryta, yielded a fraction to which they gave the name *oryzanin.* The crude oryzanin obtained by this process was purified by precipitation with tannic acid, and from this a picrate was obtained which was crystallized, by evaporation from acetone, in small yellow-brown needles. These, on decomposition, yielded a very active curative substance. After boiling two hours with 3 per cent hydrochloric acid, the crude oryzanin yielded choline, glucose, and two acids, $C_{10}H_8NO_4$ and $C_{18}H_{16}N_2O_9$. Both acids are difficultly soluble in cold water, somewhat more soluble in hot water, readily soluble in alkali and alcohol. Both give an intense diazo reaction with p-diazobenzol-sulphonic acid, a deep indigo blue with phosphomolybdic acid and ammonia, a

[82] **Funk,** J. Physiol. **46,** 173, 1913.
[83] **Suzuki, Shinamura and Odake,** Bioch. Ztschr. **43,** 89, 1912.

strong Millon reaction, and decolorize iodine-starch solution. These reactions are also given by crude oryzanin.[83a]

Drummond and **Funk**[84] repeated this work but were not able to confirm it. Neither were the above mentioned acids detected, nor was a curative picrate obtained. On the contrary it was found that the vitamin was not precipitated by picric acid. It was hoped that by using large quantities of rice polishings positive results could be obtained. In the first instance 380 kilos of rice polishings were used and the method carried out exactly as described above. In another case an extract from 620 kilos of rice polishings was worked up. This extract was hydrolyzed with acid previous to fractionation. In both cases a most careful inquiry failed to detect any new chemical substance that could be regarded as the vitamin itself or a decomposition product of it.

Funk[85] concluded that the vitamin is extremely unstable and that methods employed in separating are likely to destroy it largely if not completely. Of the various destructive agencies he regarded the action of alkali as probably the most important and therefore endeavored to find a satisfactory method of separation, which would avoid the use of alkali. He found[86] that the phosphotungstic precipitate from alcoholic extract of yeast can be divided by means of acetone into a small insoluble fraction which contains the bulk of the vitamin, and a large soluble fraction which is totally inactive. By using lead acetate instead of baryta to decompose the phosphotungstates very clear solutions are obtained which facilitate further purification and the possible destructive action of the alkali is eliminated.

Recognizing the same difficulty in the use of alkali, **Vedder** and **Williams**[87] and **Sullivan** and **Voegtlin**[88] sought to avoid it by use of barium acetate and lead acetate respectively to decompose the phosphotungstic precipitates, and report some measure of success.

Pol[89] separated a crystalline acid which he assumed to be the active principle from a kind of bean, the katjang idjoe (Phaseolus radiatus) which was used by **Grijns**[90] for the cure of polyneuritis.

[83a] **Kaoliang** (Andropogon sorghum, Brot.) is reported by **Kimura** (J. S. C. I. 1921, 672 A) to be rich in orizanin.

[84] **Drummond and Funk**, Bioch. J. 1914, 8, 598.

[85] **Funk**, Bioch. Bull. 5, 1, 1916.

[86] **Funk**, Bioch. Bull. 5, 1, 1916.

[87] **Vedder and Williams**, Phil. J. Sci. 1913, 8, 175, 183.

[88] **Sullivan and Voegtlin**, J. Biol. Chem. 1916, xxiv.

[89] **D. J. Hulshoff Pol**, J. Physiol. 51, 432, 1917.

[90] **Grijns**, Geneesk, Tyds. v. Ned. Indie, 41, 1901.

For the isolation of this acid the beans were boiled in excess of water, the fluid was decanted and basic lead acetate added. The precipitate was collected on a filter, well washed with water, and then water added to it till it formed a thin pap. Through this hydrogen sulphide was passed to remove the lead, and carbon dioxide was bubbled through the filtrate to remove the hydrogen sulphide. The fluid thus obtained was administered to four out of a number of beriberi patients, all of whom recovered. To isolate the active principle further, the purified extract was evaporated to dryness and crystals obtained. Both the extract and the crystals were acid. The constitution of the acid was not determined.

Suguira[91] prepared a colorless, crystalline substance capable of exerting a curative action on polyneuritic pigeons from dried, powdered brewers' yeast by treating it with ten times its weight of 5 per cent sodium chloride solution, and subjecting it to air dialysis. By this method, 22 mg. of colorless, crystalline substance, almost free from sodium chloride, can be obtained from 10 grams of dried yeast. A solution in water of from 2 to 5 mg. of these colorless crystals injected subcutaneously into a pigeon may be sufficient to remove completely the symptoms of polyneuritis within a few hours. Its constitution was not determined.

Hofmeister[92] isolated an antineuritic substance from rice to which he gave the name *oridine*. It was found to be isomeric with betaine and valine and from its properties would seem to be related to the pyridine and piperidine groups.

The hydrochloride was prepared as follows: Rice meal was extracted with 80 per cent alcohol, the alcoholic extract evaporated to a syrup in a vacuum, and made up with hydrochloric acid to a solution containing 3 per cent of the acid. After extraction of impurities with ether, the acid solution was concentrated again to a syrup and made feebly alkaline with sodium carbonate, and precipitated with bismuth-potassium iodide solution. After acidifying the filtrate, the precipitation was repeated, the precipitate being triturated with silver carbonate and decomposed with hydrochloric acid. The substance was then obtained in crystalline form on drying the filtrate. Doses of 5 to 10 mg. of the hydrochloride were found to be active. The aurichloride, $C_5H_{11}O_2N,HAuCl_4$, formed platelets and flat prisms, m. p. 277°. *Oridine hydrochloride,* $C_5H_{11}O_2N,HCl$, forms colorless prisms, m. p. 240°. The free base was a white, hygroscopic powder, soluble in water. On purification the compound lost its activity.

[91] **Suguira, J. Biol. Chem. 36, 191, 1918.**
[92] **Hofmeister, Bioch. Ztschr. 1920, 103, 218.**

Myers and **Voegtlin** [93] have recently obtained an active crystalline compound from autolyzed yeast extract by a modification of **Funk's** method which eliminates purines, histidine, proteins and albumoses. Their procedure is as follows:

Air-dried yeast is ground to a fine powder and twice extracted for three hours with boiling methyl alcohol, containing 0.01 per cent hydrochloric acid, in the proportion of 2 c. c. of alcohol to each gram of yeast. The alcohol is removed in vacuo at 35° C. and the residue repeatedly extracted with ether and 0.1 per cent hydrochloric acid, keeping the volume as small as possible. From this acid extract the purines are precipitated by hot aqueous silver acetate, very little of the active principle being carried down in this fraction. To the filtrate, containing a large excess of silver acetate, saturated barium hydroxide solution is added to precipitate the vitamins along with other extraneous material. This precipitate is suspended in water, acidified with sulphuric acid, and decomposed with hydrogen sulphide, the filtrate treated with slight excess of lead acetate to remove the sulphuric acid, and again with hydrogen sulphide to remove the lead, then concentrated in vacuo. Up to this stage, according to Myers and Voegtlin, practically none of the activity is lost. On addition of mercuric sulphate to the concentrated vitamin solution an inactive histidine fraction is precipitated. The filtrate containing an excess of mercuric sulphate is treated with absolute alcohol until precipitation is complete. The pale yellow precipitate formed contains the bulk of the active material. " The degree of separation in this last procedure is influenced by two factors, (1) the concentration of excess mercuric sulphate present and (2) the final concentration of ethyl alcohol. If these adjustments are right, none of the active material remains in the alcoholic filtrate." The alcohol-insoluble material is suspended in water and the mercury removed by means of hydrogen sulphide. The filtrate from the mercuric sulphide is freed of hydrogen sulphide in vacuo and the sulphuric acid removed by means of lead acetate and the excess of lead by hydrogen sulphide. By concentration in vacuo over soda lime a definitely crystalline product was obtained by Myers and Voegtlin, which crystallized in spindle-shaped crystals and was active as long as the crystals were surrounded by the mother liquor. When washed with absolute alcohol and dried, the crystals lose their activity and change from spindles to prisms, but on recrystallization from water, they take on the spindle shape again. The investigators believe

[93] **Myers and Voegtlin,** J. Biol. Chem. 42, 199, 1920.

that there are at least two substances in the final solution, both possessing a distinctly basic character.[93a]

Seidell[93b] has found that the precipitate obtained by addition of ammoniacal silver nitrate to a purified vitamin extract made from yeast " activated " fuller's earth is highly antineuritic. This vitamin silver complex is amorphous and its conversion to a crystalline condition has not been effected, but several crystalline derivatives of the active constituent of the compound have been obtained, including the picrate, nitrate, and what appears to be the free base. Of these, the picrate does not give a constant melting point and yields picric acid by ether extraction. The nitrate melts with decomposition at 146°. The base is very slightly soluble in strong alcohol but so soluble in water that a viscous pellicle is usually obtained on slow evaporation of the aqueous solution.

Funk[94] at first believed that the antineuritic vitamin was a pyrimidine base analogous to uracil acid and thymine, and was probably a constituent of nucleic acid; a theory which was supported by **Vedder** and **Williams.**[95] It seems evident, however, from later work,[96] that the substances isolated have in all cases been nitrogeneous bases contaminated by traces of vitamin, rather than the vitamin itself in a pure condition. Antineuritic properties have been attributed to a variety of compounds; nicotinic acid,[97] adenine,[98] betaine,[99] allantoin,[100] pyrimidines,[101] hydroxy pyrimidines,[102] to certain purine and choline-like bases isolated from rice polishings;[103] to quinine and

[93a] Yeast extract does not cause secretion of pancreatic juice as does secretin. Secretin can be extracted from the intestine of a cat showing the so-called polyneuritic condition to a very marked degree. Vitamin *B* has a curative effect on polyneuritic animals when given by the mouth, whereas secretin has no action on the secretion of pancreatic juice when so administered. The suggestion of Voegtlin and Myers that vitamin *B* and secretin are identical is not supported is the conclusion reached by **Anrep** and **Drummond** (J. Physiol. 54, 249, 1921).

[93b] **Seidell,** Public Health Reports, April 1, 1921; Science, Aug. 26, 1921, 177.

[94] **Funk,** J. Physiol. 45, 75, 1912.

[95] **Vedder** and **Williams,** Phil. J. Sci. B8, 175, 1913.

[96] **Drummond** and **Funk,** Bioch. J. 1914, 8, 598; **Barger,** "The Simple Natural Bases," [Longmans] 1914, 112.

[97] **Funk,** J. Physiol. 1913, 46, 177.

[98] **Williams** and **Seidell,** J. Biol. Chem. 1916, 26, 431.

[99] **Funk,** J. Physiol. 45, 489, 1912-13.

[100] **Funk,** *Ibid.*

[101] **Funk,** J. Physiol. 45, 489, 1912-13; **Williams,** J. Biol. Chem. 29, 504, 1917.

[102] **Williams,** J. Biol. Chem. 29, 504, 1917.

[103] **Vedder** and **Williams,** Phil. J. Sci. 8B, 175, 1913.

cinchonine,[104] and to thyroxin, desiccated thyroid gland, pilocarpine hydrochloride, and tethlin,[105] but in many cases when these substances are prepared in sufficiently pure condition they have proved to be inactive,[106] and in no case has the vitamin been satisfactorily identified with a known substance. The work of **Williams** along this line seemed very promising, but unfortunately other workers have been unable to confirm his results.

Assuming from the properties of the active extracts that the curative substance was an hydroxy-pyrimidine, **Williams**[107] prepared a series of pyrimidine derivatives and tested the therapeutic action of each on polyneuritic pigeons. The series included nicotinic, cinchomeric, quinolinic, 6-hydroxy-nicotinic, and citrazinic acids, α-hydroxypyridine glutazine, 2, 3, 4-trihydroxypyridine, and its anhydride, 2, 3, 4-trihydroxypyridine, and tetrahydroxypyridine, of which only α-hydroxy-, 2, 4, 6-, and 2, 3, 4-trihydroxypyridine showed any curative effect. As it was noticed that preparations which were active when fresh deteriorated rapidly on keeping,[108] Williams suspected isomerization. He was able to prepare two isomeric crystalline forms of hydroxy-pyridine, needles and granules, which are isomeric and can be converted into one another, the stability of each form being determined by temperature, solvent and other conditions. Only the needle form shows curative power. On standing at room temperature the needles gradually become transformed into granules, with corresponding loss of curative power. He concluded that the curative form is a pseudo-betaine and that more or less conformity to the betaine type in structure or energy conditions is an essential characteristic of antineuritic vitamins, and suggested that the curative properties of **Funk's** vitamin fractions of yeast and rice may have been due in part to a corresponding isomeric form of nicotinic acid or a polymer or simple derivative of it.[108a]

Williams and **Seidell**,[109] in their second paper on the subject,

[104] **Cooper**, Bioch. J. **7**, 268, 1913.

[105] **Dutcher**, J. Biol. Chem. **39**, 63, 1919.

[106] **Drummond** and **Funk**, l. c.; **Voegtlin** and **White**, J. Pharm. Exp. Ther. 1316, **9**, 155; **Harden** and **Zilva**, Bioch. J., 1917, **11**, 172; **Steenbock**, Proc. Am. Physiol. Soc. Am. J. Physiol. **42**, 610.

[107] **Williams**, R. R., J. Biol. Chem. 25, 437, 1916.

[108] **Williams**, R. R., J. Biol. Chem. 29, 495, 1917.

[108a] R. R. **Williams** believes that vitamin *B* eventually will be found to be a cyclic nitrogen compound with an oxygen substitution in the ring and capable of existence in a betaine configuration. (J. Ind. Eng. Chem. 1921, 1108.)

[109] J. Biol. Chem. **26**, 431, 1916.

give expression to the following hypothesis: The vitamins contain one or more groups of atoms constituting nuclei in which the physiological properties are resident. In a free state these nuclei possess the vitamin activity but under ordinary conditions are spontaneously transformed into isomers which do not possess the antineuritic power. The complementary substances or substituent groups with which these nuclei are more or less firmly combined in nature exert a stabilizing and perhaps otherwise favorable influence on the curative nucleus, but do not in themselves possess the vitamin type of physiological potency. Accordingly it is believed that while partial cleavage of vitamins may result only in a modification of their physiological properties, by certain means disruption may go so far as to effect a complete separation of " nucleus " and " stabilizer " and, if it does so, will be followed by loss of curative power due to isomerization. The basis for the assumption that an isomerization constitutes the final and physiologically most significant step in the inactivation of a vitamin is found in studies of synthetic antineuritic products, and is supported by evidence of the existence of such an isomerism in the crystalline antineuritic substance obtainable from brewers' yeast. **Williams** holds that natural antineuritic substances, from the same or different raw materials may differ both in respect to the ease with which the stabilizing groups or substances are detached and to the rapidity with which the resulting free curative nucleus is transformed into a non-curative isomer. The acceptance of this hypothesis does not predicate anything with regard to the number or identity of the curative nuclei in the various antineuritic feeding stuffs nor does it negative the possibility of an actual disruption and decomposition of the vitamin molecules as a contributory cause of their stability.

Harden and **Zilva**[110] question the view that hydroxypyridine cures or improves the condition of polyneuritic birds. They confirm the existence of two isomeric forms of this compound, but failed to observe any curative action of either form. **McCollum** and **Simmonds**[111] do not agree that the needs of the animal are for a specific type of labile isomerism rather than a specific chemical complex, and suggest that the temporary relief of polyneuritis by so many unrelated substances, as noted above, may be the result of the pharmacological action of these substances resulting in the stimulation of certain of the cells of the body rather than renewed function of cells which have been subjected to a selective fast and later have been supplied with the missing food complex. In support of this view they

[110] **Harden and Zilva, Bioch. J., 11, 172, 1917.**

[111] **McCollum and Simmonds, J. Biol. Chem. 33, 55, 1918.**

point out that histological methods have shown polyneuritic animals to suffer a degeneration of the motor cells of the cord, and that this change is progressive, some cells presenting the normal appearance while others in the same field are degenerated.

"It would appear plausible that when the motor cell changes have reached a certain point loss of function supervenes and paralysis results. There still remain, in the cord of a paralyzed animal, motor cells which appear normal when stained and which may be capable of restoring the motor functions of the muscles when influenced by substances which stimulate them to heightened sensitiveness. . . . If this line of reasoning could be shown to be valid, it would follow that experiments with pure chemical substances of known constitution, with a view to finding by good fortune the one playing an important physiological rôle, might be entirely misleading unless it were shown that the 'cure' was permanent. For such complete proof it is necessary to demonstrate the resumption of growth and maintenance of health as long as the substance is supplied in the food mixture. . . . Sustained normal function is indispensable to adequate proof that the dietary essential in question is being administered."

While we are still far from a definite conclusion as to the chemical structure of the vitamin, there are certain properties in addition to those already referred to which are invariably associated with the physiologically active substance. It appears to be basic,[112] although **Osborne, Wakeman** and **Ferry** [113] suggest that the persistence with which it is retained by edestin may indicate that it is chemically combined with the edestin, and hence that it has acid properties. It is completely precipitated by silver nitrate and barium hydroxide,[114] by phosphotungstic acid in 5 per cent sulphuric acid solution,[115] giving a phosphotungstate which is insoluble in acetone,[116] and almost entirely by ammonium molybdate.[117] It can be precipitated by tannic acid,[118] but this method when applied to rice polishings does not yield large amounts.[119] **Drummond** [120] found that upon fraction-

[112] **Funk,** Trans. Soc. Tr. Med. 5, 86, 1911; J. Physiol. 43, 395, 1911; **Vedder,** Phil. J. Sci. 7B, 415; **Osborne** and **Wakeman,** J. Biol. Chem. 40, 383; **Myers** and **Voegtlin,** J. Biol. Chem. 42, 199, 1920.

[113] **Osborne, Wakeman** and **Ferry,** J. Biol. Chem. 39, 35, 1919.

[114] **Funk,** J. Phys. 43, 395, 1911; **Cooper,** Bioch. J. 7, 268, 1913; **Edie, Evans, Moore, Simpson** and **Webster,** Bioch. J. 6, 234, 1912.

[115] **Cooper** and **Funk,** Lancet, 1911, ii, 1266; **Funk,** J. Physiol. 43, 395, 1911; 45, 75, 1912; **Funk** and **Macallum,** J. Biol. Chem. 27, 63, 1916; **Eustis** and **Scott,** Bioch. Bull. 3, 466, 1914.

[116] **Funk,** Bioch. Bull. 5, i, 1916; **Drummond,** Bioch. J. 11, 255, 1917.

[117] **Cooper,** J. Hyg. 12, 436, 1913.

[118] **Suzuki, Shinamura** and **Odake,** Bioch. Ztschr. 43, 89, 192.

[119] **Vedder** and **Williams,** Phil. J. Sci. B8, 175.

[120] **Drummond,** Bioch. J. 11, 255, 1917.

ation of yeast dialysate with silver nitrate and baryta, the precipitate produced with silver nitrate, which contained the purine fraction, had no effect on growth, while that produced on the further addition of baryta and containing the pyrimidine contained small traces of vitamin.

It is partially precipitated by mercuric chloride [121] but not at all by basic lead acetate,[122] platinic chloride,[123] or picric acid.[124]

With the phosphotungstic acid solution introduced by **Folin** and **Macallum** [125] as a test for uric acid it gives a deep blue color,[126] and a similar color is obtained with the phosphomolybdic-phosphotungstic acid reagent of **Folin** and **Denis.**[127] **Eddy** [128] believes from his experiments that this color reaction offers a practical method of standardizing dosage.

The "uric acid reagent" has been found to give a reaction with di- and polyhydric phenols, monohydric phenols containing an NH_2 group in the benzene ring, and certain purine and tyrosine derivatives, while the phosphomolybdic reagent appears to be specific for purine derivatives and polyphenols. Amino acids, polypeptides, and diketopiperazines are negative to both reagents. The replacement of one hydrogen atom in the purine ring lessens or abolishes the uric acid reaction. In the case of phenol reagent this occurs when two hydrogen atoms are replaced.[129] Referring to the color produced by the **Folin-Macallum** reagent, **Williams** and **Seidell** [130] state:

> It has been our experience that a negative test for this color indicates the absence of the curative substance in crude solution. A positive test can with less assurance be taken to indicate the presence of the vitamins.

From its frequent association with lipoid and phosphorus-containing compounds[131] it was natural to expect that the anti-

[121] **Funk,** J. Physiol. 43, 395, 1911; Bioch. Bull. 5, 1, 1916.

[122] **Cooper,** J. Hyg. 12, 436, 1913; **Vedder** and **Williams,** Phil. J. Sci. B8, 175, 1913; **Funk,** Bioch. Bull. 5, 1, 1916.

[123] **Funk,** l. c.; **Suzuki** and **Shinamura,** J. Tok. Chem. Soc. 32, Zentr. Bioch. Biophys. 12, 11; **Suzuki, Shinamura** and **Odake,** l. c.; **Steenbock,** Proc. Am. Physiol. Soc., Am. J. Physiol. 42, 610, 1917.

[124] **Funk,** l. c.; but cf. **Suzuki, Shinamura** and **Odake,** l. c.

[125] **Folin** and **Macallum,** J. Biol. Chem. 11, 265; 13, 363; **Benedict** and **Hitchcock,** *Ibid*. 20, 619, 1915.

[126] **Funk** and **Macallum,** Bioch. J. 7, 356, 1913.

[127] **Folin** and **Denis,** J. Biol. Chem. 12, 239; **Suguira,** J. Biol. Chem. 1918, 36, 191.

[128] **Eddy,** Proc. Soc. Exp. Biol. Med. 14, 164, 1917.

[129] **Funk** and **Macallum,** Bioch. J. 1913, 7, 356.

[130] **Williams** and **Seidell,** J. Biol. Chem. 26, 431, 1916.

[131] **Fraser** and **Stanton,** Studies from the Inst. of Med. Res. Fed. Malay

neuritic vitamin itself might be a phosphorus compound, but this appears to be disproved by the work of **Teruuchi**,[132] who showed that only 0.001 per cent of the total phosphorus of the rice grain is present in the alcoholic vitamin extract, and this presumably inorganic phosphorus, and of **Chamberlain** and **Vedder**, who found from analysis[133] the following composition for the antineuritic extract from rice: total solids, 1.34 per cent; ash, 0.03 per cent; phosphorus pentoxide, 0.00.65 per cent; nitrogen, 0.040 per cent; sucrose, 0.88 per cent. **Suzuki** and **Shinamura** found[134] nitrogen and sulphur, but no phosphorus in the active substance which they isolated from rice grits. From the extract they isolated an acid which when heated with 2 per cent hydrochloric acid gave yellow needles or prisms having a melting point 300° C. These crystals gave the Millon and the Ehrlich diazo reactions. The liquid filtered from the crystals strongly reduced Fehling solution. From these properties the acid is supposed to be a glucoside.

Voegtlin[135] claims that although phosphorus is not known to be a constituent of vitamins yet a fairly accurate index of the vitamin content of cereals may be given by their phosphorus content.

Little or no attempt has been made up to the present to isolate the antiscorbutic factor in a pure state. It is soluble in water and dilute alcohol;[136] it can be dialyzed through parchment,[137] and passed through a Berkefeld filter without losing[138] its antiscorbutic activity to any appreciable extent. It is not absorbed by fuller's earth or dialyzed iron; a property which makes it[139] possible to separate the antineuritic and antiscorbutic vitamins quantitatively from a mixture of the two by selective absorption.

Harden and **Zilva** found that the antiscorbutic[140] vitamin of

---

States 1909. Etiology of beriberi; **Cooper**, Bioch. J. 1914, 8, 347; **Voegtlin** and **Myers**, Pub. Health Repts. 33, 23, 1918, p. 911; **Sullivan** and **Voegtlin**, Proc. Soc. Biol. Chem. 1915, J. Biol. Chem. 34, xvii, 1915-16.

132 **Teruuchi**, **Saikingakuzashi**, Tokio, 1910, No. 179, Zentr. Bioch. Biophys. 11, 719.

133 **Chamberlain** and **Vedder**, Phil. J. Sci. 6, 395, 1911.

134 **Suzuki** and **Shinamura**, J. Tok. Chem. Soc. 32, Zentr. Bioch. Biophys. 12, 11.

135 **Voegtlin**, J. Wash. Acad. Sci. Oct. 4, 1916; **Voegtlin** and **Myers**, Publ. Health Repts., 33, 23, 1918.

136 **Holst** and **Frölich**, Ztschr. Hyg. 75, 334, 1913; **Harden** and **Zilva**, Bioch. J. 12, 93, 1918; **Hess** and **Unger**, J. Biol. Chem. 35, 479, 1918.

137 **Holst** and **Frölich**, Zeitschr. f. Hyg. u. Infectionsk. 72, 1, 1912.

138 **Harden** and **Zilva**, l. c.

139 **Harden** and **Zilva**, l. c.

140 **Harden** and **Zilva**, Lancet 1918, ii, 320.

orange juice was destroyed by dilute alkali at room temperature. Orange juice made neutral to phenolphthalein and given in doses of 3, 5, or 7 cc. kept the animals in good condition for 78 days, and when they were killed at the end of this time the post-mortem showed no signs of scurvy. When, however, the orange juice was made N/20 alkaline with sodium hydroxide and stored for 24 hours in a cold room these doses did not prevent or delay the onset of scurvy. If this alkaline juice was made N/20 acid with HCl just before administration the effect was still the same. Another set of animals received orange juice made N/50 alkaline with NaOH on the day of feeding. All died of scurvy after a slightly prolonged period, thus showing that the juice after being made alkaline retained only a trace of its antiscorbutic activity. It has been pointed out that many antiscorbutic vegetables are either neutral or very slightly acid, and any culinary operation which entails alkaline treatment even to a slight degree will be instrumental in the destruction of a significant part, at least, of this factor. **Hess** and **Unger**[141] note that after canned tomato and orange juice had been rendered 0.05N alkaline to phenolphthatein, these juices were still effective if administered shortly after alkalinization. If, however, 24 hours elapsed between the alkalinization and feeding then a considerable amount of the antiscorbutic factor was lost. **Delf**[142] obtained indications that the remarkable resistance of the antiscorbutic factor of orange juice to the destructive effect of heat is due to something other than the acid content of the juice, since the stability persisted even when the juice was nearly neutralized before heating.

In this connection the work of **McClendon** and **Sharp**[143] on the hydrogen ion concentration of foods during storage and preparation is of interest in relation to preservation of antiscorbutic properties.

Dutcher, Harshaw and **Hall**[144] reach the following conclusions: The antiscorbutic vitamin is not destroyed by heating at pasteurization temperature (63° C.) for 30 minutes in closed vessels or by boiling (100° C.) for 30 minutes under reflux condensers. Hydrogen peroxide possesses some destructive action when added to orange juice at room temperature and the destructive action is increased when the orange juice-hydrogen peroxide mixture is heated at 63° and tion but, in the absence of oxidizing agents, are stable to heat up to the boiling 100° C. The antiscorbutic properties of orange juice are susceptible to oxidatemperature of orange juice.

[141] **Hess** and **Unger**, J. Biol. Chem. 38, 293, 1919.
[142] **Delf**, Bioch. J. 1920, 14, 211.
[143] **McClendon** and **Sharp**, J. Biol. Chem. 41, iv. 1920.
[144] **Dutcher, Harshaw** and **Hall**, J. Biol. Chem. 47, 483, 1921.

The antineuritic factor in autolysed yeast and the antiscorbutic factor in decitrated lemon juice diffuse through a collodion membrane of such permeability as permits the passage of substances such as Methylene Blue, Neutral Red, and Safranine. It is suggested by **Zilva** and **Miura**[145] that the active molecules whether simple or associated may be of a semi-colloid nature.

Sherman, LaMer and **Campbell**[146] found that in the case of canned tomato juice the rate of destruction in one hour at 100 C., increased progressively with decreased hydrogen concentration. If the material was acidified immediately after the heating the deterioration was less than when this was omitted, pointing to a destructive effect of the hydroxyl ion even at lower temperatures.

The juices of green malt, carrots, cabbage, potatoes, turnips, and lean beef were all found to be distinctly acid whether fresh, or after cold storage, or after boiling in an open vessel. The juice pressed out of boiled food was acid and the juice boiled after pressing out of fresh food was acid. The unboiled preparations became more acid on standing but the boiled preparations were so constant as to indicate that their content of volatile acids or bases was very low. The fact that acid fruit and vegetable juices seemed to retain their antiscorbutic properties longer than neutral or alkaline extracts[147] led **Holst** to the conclusion that the antiscorbutic vitamin was more stable in acid media, and the suggestion has been made[148] that the water in which vegetables are boiled should be rendered slightly acid with citric acid in order to lessen the loss of antiscorbutic power during cooking. Evidence as to the efficacy of such treatment is conflicting however. **Holst** and **Frölich**[149] found that whereas freshly expressed cabbage juice lost its antiscorbutic power completely after heating from 60° to 100° C. for 10 minutes, if the juice was acidified with 0.5 per cent citric acid before heating, some antiscorbutic power was retained, but **Delf**[150] failed to confirm this and states:

> "There should be no addition of either alkali or acid to the water (in which vegetables are boiled) as this increases the inevitable loss of antiscorbutic vitamins."

[145] Biochem. J., 1921, 15, 422.

[146] Sherman, LaMer and Campbell, Sci. Aug. 26, 176, 1921.

[147] **Holst** and **Frölich**, Norsk. Mag. Lagev. 1910, No. 3, Zeitschr. Hyg. 72, 1, 1912; **Hess** and **Unger**, Proc. Soc. Exp. Biol. Med. 1918, 15, 141, 16, 1; **Givens** and **McClugage**, Proc. Soc. Biol. Chem. J. Biol. Chem. 41, xxiv, 1920.

[148] **Grieg**, E. D. W. quoted by **Delf**, Bioch. J. 1918, 12, 420.

[149] **Holst** and **Frölich**, Ztschr. Hyg. 72, 1, 1912.

[150] **Delf**, Bioch. J. 12, 431.

Holst and **Frölich** (l. c.) themselves noted that the juice of dandelion leaves was no more thermostable when acidified than in its natural condition, and sorrel leaf juice which is acid in reaction when expressed, lost all its protective action when heated. **Givens** and **McClugage**[151] found that the antiscorbutic power of potatoes is lessened by soaking in dilute hydrochloric or acetic acid before cooking. Cooking for 15 minutes in weak solution such as 0.5 per cent citric acid did not destroy the antiscorbutic vitamin.

Although crude uncrushed potato exercises a marked antiscorbutic action, its juice extracted by pressure possesses only a feeble action.[152] If, however, the potato is mixed with 2½ per cent. by weight of a mixture of 1 part of citric or tartaric acid and 4 parts of sucrose before being crushed and the juice expressed, such juice possesses a much more marked antiscorbutic action. The loss of antiscorbutic properties of the potato by simple pressure is considered to be due to the action of oxidases during the process, and this action is inhibited by the presence of citric acid or tartaric acid. The juice from fresh potatoes has a stronger antiscorbutic action than that from potatoes stored during the winter.

A test advocated by **Bezssonoff**[153] to determine the presence of antiscorbutic vitamin is the following. A modified Folin-Denis phenol reagent prepared by adding an equal volume of N/1 sulphuric acid to a solution containing 100 g. of sodium tungstate, 20 g. of phosphomolybdic acid and 16.6 c.c. of concentrated phosphoric acid per litre, gives a blue coloration with plant extracts known to possess antiscorbutic properties, but not with extracts devoid of these properties. Of the various phenols examined the only one to give this blue color with the reagent was quinol.

[151] **Givens** and **McClugage**, J. Biol. Chem. **42**, 511, 1920.

[152] **Bezssonoff**, Comptes rend, 1921, **173**, 417; J. S. C. I., 1921, 671A; see also J. S. C. I., 1921, 126A.

[153] **Bezssonoff**, Comptes rend., **173**, 466, 1921.

CHAPTER VII

CONCENTRATED PREPARATIONS OF VITAMINS

Various methods have been devised for obtaining preparations of vitamins in concentrated form for use where the fresh foodstuffs are not available or where it is desirable to eliminate every variable in nutritive factors except the one under consideration.

The first concentrate of *A* was the "butter oil"[1] prepared by **Osborne** and **Mendel**,[2] but this was found to deteriorate on keeping, even under the best conditions, so that within a year its efficacy was almost entirely lost. A more satisfactory preparation was obtained from dried plant tissues by extracting with ether. The oily residue obtained when the ether was distilled amounted to about three per cent of the weight of the dried plant. Most of these oils were potent in stimulating growth. A striking exception was found in the extract of dried tomato which was not effective, although the dried tomato itself gave the best growth obtained in the whole series of experiments carried out at that time. The great variation in the ease with which different plant tissues yield their vitamin to solvents has already been noted,[4] and apparently the grasses, clover, alfalfa, and timothy, and spinach are particularly favorable sources for this type of preparation.

Zilva[5] prepared alcoholic extracts of *A* from fresh cabbage and carrots by allowing the finely triturated foodstuff to stand for 12 to 18 hours with alcohol, in the proportion of 100 g. of foodstuff to 500 c. c. of alcohol. This was then filtered and the filtrate evaporated in vacuo at 35° C. The residue is said to be effective in promoting growth. **Steenbock** and **Boutwell**[6] regard alcohol as the most satisfactory solvent for the extraction of *A* from plant products, having found it considerably more effective than ether in this respect. Benzene also proved fairly satisfactory although inferior to alcohol.

Since *A* is not destroyed by drying at moderate temperatures, dried

[1] **Osborne and Mendel**, J. Biol. Chem. 41, 549, 1920.

[2] See p. 90.

[4] See pp. 111, 112.

[5] **Zilva**, Bioch. J. 14, 494, 1920.

[6] **Steenbock and Boutwell**, J. Biol. Chem. 42, 131, 1920.

plant products may be used as a convenient source of supply. **Osborne** and **Mendel**[7] found 0.1 gram of dried alfalfa, clover, timothy, or spinach as effective as an equal quantity of butter-fat, while dried tomato was still better. The products used were prepared from the plant materials by heating in a large drier, through which a current of air circulated at 60° C. or less, and grinding the dry residues to a powder.

In making an extract of *A*, **McClendon**[7a] uses green leaves or fruit skins. These are dried, preferably in the absence of oxygen, and then ground to a powder. The powder is moistened with alcohol by being thrown into a container half filled with boiling 95 per cent alcohol and allowed to remain without application of heat for twenty-four hours. The mass is then placed in a strong canvas bag and subjected to a pressure of 5000 lbs. to the square inch. The press-cake may be ground in a mill and re-extracted if desired in the same manner. If, however, it is desired to recover the solvent, a preliminary concentration may be carried out in a vacuum pan. The product is a sticky powder when absolutely dry, but it absorbs water from air and becomes pasty. It contains resinous or fatty substances. When made from spinach a hygroscopic substance of waxy constituency is obtained. This preparation of *A* contains sufficient fat-soluble vitamin to afford normal growth in a rat, when 0.05 to 0.1 gram is added daily to a ration free from this factor.

The methods which have been used for preparing concentrates of vitamin *B* fall into three classes: (1) those in which an extract of the food substance is evaporated to dryness at low temperature; (2) those in which the vitamin is precipitated from an extract containing it, the precipitate after purification is decomposed, and the resulting solution evaporated in vacuo; and (3) those in which the vitamin is absorbed by fuller's earth or some similar absorptive agent and the "activated" product thus obtained used without further treatment.

The first method of procedure was used by **Bosshard** and **Hefti**,[8] who treat substances rich in vitamins, particularly yeast or rice bran, with dilute mineral acids at 80° C. until the biuret reaction gives a negative result. The product is filtered, freed from mineral acid, and then evaporated to dryness at the lowest possible temperature, either without further treatment, or after the conversion of the amino acids present into their calcium or sodium salts. By avoiding too

[7] **Osborne and Mendel**, J. Biol. Chem. 41, 549, 1920.

[7a] **McClendon**, J. Biol. Chem. 1921, 411.

[8] **Bosshard and Hefti**, J. S. C. I., 1920, 675A; German Patent 320,785, March 19, 1916.

high a temperature or acid concentration, the vitamins are said to be brought unchanged into solution.

In a process recommended by the Soc. anon. pour l'ind. chim. A. Bâle,[9] vitamin preparations are made by comminuting vegetable substances so as to retain the juice, adding a small proportion of acid, if necessary, so as just to neutralize any alkali that may be present, and drying in vacuo at a temperature below 100° C. Preparations of this sort can be baked into loaves by adding flour and a distending agent such as sodium bicarbonate or yeast.[9a]

Voegtlin[10] gives the following method for the extraction of vitamin *B* from animal tissues:

Extract from ox liver.—Fresh ox liver was minced in a meat chopper and dried, first on glass plates at about 40° C. in a current of air from an electric fan, and then in a vacuum desiccator. The dried material was then ground to a fine powder and extracted with 95 per cent alcohol for several days at 37° C. About two liters of alcohol were used for each kilo of dried material. One half of the alcohol was used for the first extraction, and the remainder for the second extraction. During the extraction the bottles containing the material were occasionally shaken. The alcohol was then filtered off and evaporated in vacuo at 35 to 40° C., until the residue in the flask showed a tendency to form viscous bubbles which rise in the flask. The viscous residue was then poured into a separatory funnel and extracted with about twice its volume of ether. Three distinct layers formed, upon allowing the contents of the funnel to stand for three hours. The upper layer consisted of an ethereal solution of fats and lipoids, the middle layer of a white or yellowish insoluble residue, and the lower layer of a yellowish or brown colored oil-like substance. The material contained in the lower layer will hereafter, for the sake of brevity, be designated as the "vitamin" fraction. The "vitamin" fraction was then run off into another separatory funnel, containing ether, and thoroughly agitated. After it had settled out, the oily material was run off into a dish and the ether evaporated off in vacuo. The yellowish oil had a pleasant, nutty odor, and was soluble in water. **Cooper** states that this material is soluble in absolute alcohol, but it was found that its solubility was very slight in absolute alcohol; whereas 50 per cent alcohol dissolves it quite readily. In case a sharp separation of the various layers was not produced upon extraction with ether, as much oily material as possible was run off, the mixture of oil and solid material was run into a dish, freed from ether and the mixture extracted with a small amount of 50 per cent alcohol. After filtering, the alcoholic solution was again poured into ether and the lower layer was treated as above. The various vitamin fractions were then united and the ether evaporated in vacuo.

Extract from thymus gland.—In the preparation of the vitamin fraction from the thymus gland of the hog, the following changes were found to be

[9] British Patent 133,183, Oct. 15, 1918.

[9a] Synthetic fats, prepared by combining higher fatty acids with glycerine, may have vitamins added thereto. (**G. Schicht** Akt. Ges., British Patent 160,840, Mar. 31, 1921).

[10] **Voegtlin, Neill and Hunter,** Hyg. Lab. Bull. 116, 1920.

necessary in the above method. Thymus glands could not be ground and dried in the usual way on account of the difficulty presented by the large amount of connective tissue. The glands were therefore cut up with scissors into small pieces and allowed to stand for several days in 95 per cent alcohol; for 6,000 grams of glands six liters of alcohol were used. The alcoholic extract was then filtered off, the residue placed in a filter press and the remainder of the liquid removed in this manner. The pressed-out thymus glands were then minced in a meat chopper and again extracted with 8 liters of 95 per cent alcohol. The filtrate from the first extraction was evaporated in vacuo. On account of the tendency to foam, the filtrate was slowly dropped into the flask by means of a long-necked separatory funnel. The weight of the sirupy residue obtained from the first extraction amounted to 120 grams. The second alcoholic extract was treated in the same manner. These fractions were then extracted with ether in a separatory funnel. The lower layer, containing the vitamin fraction, was then run off and the ether evaporated in vacuo. The greater part of the vitamin is extracted by the first treatment with alcohol. The two fractions were united and weighed 181 grams.

These extracts were, however, considerably less efficient than either activated **Lloyd's** reagent or **Funk's** fraction from rice polishings.

Funk's method of separation by means of phosphotungstic acid is described as follows: [11]

Since only relatively small quantities of vitamins are contained in the precipitate due to phosphotungstic acid, the separation of these vitamins according to this method is accomplished only with extreme difficulty. **Funk** finds by treating the phosphotungstic acid precipitate with acetone, the major portion of the precipitate, containing the inactive ingredients, may be dissolved and eliminated, leaving almost all of the vitamins in the insoluble residue of the precipitate. Thus the percentage of vitamins in the precipitate may be so increased that **Funk** claims these substances may be isolated, generally in crystalline form, after decomposing the precipitate and evaporating or concentrating the solution. Neutral lead acetate is an effective agent for decomposing the precipitate produced by phosphotungstic acid. The method may be employed for the treatment of organic extracts, such for example, as extract from the thyroid gland (scutiform glandule), to separate the active substances.

For example, 350 grams of a moist precipitate obtained from yeast extract by means of phosphotungstic acid is ground up in a mortar, together with one-half a liter of acetone, causing most of the precipitate to go into solution. The portion insoluble in acetone is separated by filtration and this residue then well washed with acetone. The residue when dried weighs about 20 grams and hence represents only a minor portion of the entire precipitate produced with the phosphotungstic acid. This insoluble residue is further treated by well mixing and grinding with a solution of neutral lead acetate and then placed on the filter, the filtrate being freed from excess of lead by treatment with hydrogen sulphide. After filtering to separate the sulphide of lead formed, the filtrate is evaporated in vacuo. The resultant product is a white substance crystallizing in the form of needles and weighing from 0.2 to 0.3 grams." [12]

[11] U. S. Patent 1,162,908.

[12] See also **Eddy,** J. Biol. Chem. 27, 113, 1916.

Gams [13] claims to have obtained much more active preparations by eliminating the phosphotungstic acid and treating directly with lead acetate in the following manner: The vitamin-containing material is extracted with dilute alcohol and the extract freed from alcohol by distillation in vacuo. The aqueous extract thus obtained is treated successively in an acid state by lead acetate and in a neutral state by basic lead acetate. The treatment with lead acetate in an acid state is claimed to produce only the precipitation of the most of the inactive ingredients, the active substance remaining in solution. By the subsequent treatment with basic lead acetate in a neutral solution, according to Gams, only further impurities are precipitated without appreciable loss of active substances. The lead is separated and by evaporating the solution in vacuo Gams secures a highly active preparation which is easily soluble in water to a clear solution and which he claims may be preserved indefinitely and adapted to be administered per se. The food products or other substances of vegetable and animal origin employed as parent materials may be subjected previously to a mechanical, physical or chemical treatment making their extraction with alcohol more easy, for instance a comminution with quartz sand, a plasmolysis, a hydrolysis with dilute acids or alkalis, or a digestion with pepsin and hydrochloric acid.

The process is illustrated by the following examples:

Rice bran in a finely-divided state is extracted, in the cold, with dilute alcohol and the solution obtained is freed from alcohol by evaporating in vacuo. The aqueous milky liquid is separated by decantation from the oil and grease floating on its surface, is acidified with acetic acid and a solution of lead acetate is added until no further precipitate is produced. The clear solution, separated by filtering off the lead acetate precipitates is accurately neutralized and a neutralized solution of basic lead acetate is added until precipitation is complete. The solution separated from the precipitate by filtering is freed from lead by means of hydrogen sulphide or sulphuric acid. In the first case, the solution obtained by filtering is directly evaporated to dryness in vacuo and in the second case the excess of sulphuric acid is removed by the addition of barium hydroxid and the solution, showing still a feeble acid reaction with Congo indicator, is concentrated in vacuo. In each case a light yellow crystalline powder is obtained, which is easily soluble in water to a clear solution and contains most of the vitamin present in the parent material.

The weak sulphuric solution, thoroughly freed from the inactive ingredients and from the lead, obtained according to the above procedure may be concentrated in vacuo and a sulphuric acid solution of mercury oxide added until no further precipitate is produced. The precipitate is filtered off, washed and treated in an aqueous supension with hydrogen sulphide. After the liquid has been separated by filtering from mercury sulphide, it is freed from hydrogen sulphide by evaporation in a vacuum or by introducing an excess of carbon-dioxide and evaporated to dryness in vacuo. The remaining residue is a yellow-brown mass, easily soluble in water, said to contain the active substances of the parent material in a concentrated and very pure form.

The solution freed from inactive ingredients and from lead, obtained according to the first example, is acidified with oxalic acid and a solution of phosphotungstic acid is added until no further precipitate is produced. The precipitate,

[13] **Gams,** British Patent 103,294, Jan. 4, 1917; Chem. Abs. 11, 1522, 1917; **Gams and Schreiber,** U. S. Patent 1,235,198, July 31, 1917.

separated by filtering and washed with water containing oxalic acid, is suspended in water and the remaining oxalic acid is precipitated with calcium carbonate. The precipitate is filtered off and the filtrate, freed from calcium compounds, is evaporated to dryness in vacuo. There is obtained a yellow-brown crystalline powder, easily soluble in water, which is said to contain the vitamin of the parent material in a highly concentrated, very pure and permanent form, and which is suitable for purposes of injection.

The use of "activated" fuller's earth was introduced by **Seidell.**[14] Washed and pressed brewers' yeast, or other source of vitamin is digested for 36 hours at 37.5° C., and the clear liquid from the resulting mass is mixed with about 50 grams per litre of finely divided fuller's earth. The mixture is shaken and treated with about 1 per cent of normal hydrochloric acid to assist settling. The sediment is washed with dilute acid and with ethyl alcohol and then dried in vacuo over sulphuric acid. The vitamins contained in the yeast extract are almost completely absorbed by the fuller's earth. The solid extract thus obtained can be taken either in liquid suspension or as capsules, about 5 grams per day being an adult dose.

The particular grade of fuller's earth found by Seidell to be most useful is a kind obtained from Surrey, England. By the autolysis of fresh yeast followed by filtration a clear reddish brown filtrate is obtained containing over 20 per cent of solids. This is very rich in vitamin. If fuller's earth is added in the proportion of 50 grams per litre and kept in intimate contact with the liquid for about half an hour and then removed by filtration, the yeast liquor is found to contain practically the same amount of solids originally present, but all of the vitamin is now firmly attached to the fuller's earth and repeated washing does not remove any appreciable amount of vitamin from it.

Physiological experiments have shown that no deterioration occurred in samples of the "vitamin-activated fuller's earth" kept over two years. Large amounts of it can be readily accumulated and after being uniformly mixed, it can be standardized by physiological tests for its vitamin content. Such material forms a particularly satisfactory starting point for the comparative study of various methods for the isolation of vitamins.

Although vitamin *B* is destroyed by heating to 120° C. for two hours, in the dry state in combination with fuller's earth it can be heated to at least 200° C. without appreciable deterioration. Various attempts have been made to remove the vitamin, in its pure form, from its combination with fuller's earth, principally by treatment with dilute alkali solutions, but all attempts have so far been unsuccessful.[14a]

Eddy and **Roper**[15] applied this method to the separation of the

[14] **Seidell,** U. S. Patent 1,173,317; J. S. C. I. 35, 653, 1916; U. S. Pub. Health Reports, 31, 364, 1916.

[14a] **Seidell,** Jour. Ind. Eng. Chem. 1921, 73. Details of experiments on the isolation of the antineuritic vitamin by silver nitrate precipitation are given by **Seidell** (J. Ind. Eng. Chem. 1921, 1111).

[15] **Eddy and Roper,** Proc. Soc. Exp. Biol. Med. 14, 52, 1916; Am. J. Dis. Child. 14, 189, 1917.

vitamin from pancreatic extract. The minced glands were extracted with 95 per cent alcohol, containing enough hydrochloric acid to make it about 0.8 per cent acid. The filtered extract was evaporated to dryness in a current of air at 25° C. The residue was extracted with water, filtered and concentrated in an air current so as to make 1 cc. correspond to 2.7 grams of pancreas. Fifty grams of Lloyd's reagent [16] were added per liter of extract, and the mixture shaken in a mechanical shaker. After standing over night the sediment was removed with the suction funnel, dried in a current of air, repowdered, and finally dried in a vacuum desiccator. When the activated Lloyd powder is dried it retains its power as a vitamin carrier, and when fed to rats exercises the same power as water solutions of vitamins.

Frankel and **Schwarz** [17] prepared an active fraction from yeast by extracting the yeast with 80 per cent alcohol, removing the fats, and treating with basic lead acetate. The lead in the active filtrate is removed with hydrogen sulphide, and the filtrate further precipitated with mercuric chloride. The precipitate is then decomposed with hydrogen sulphide, freed from hydrochloric acid, and concentrated in a vacuum. The concentrated syrup is precipitated with picrolonic acid, which removes an inactive picrolonate, and the active substance is finally precipitated with phosphotungstic acid. The precipitate is decomposed with baryta and sulphuric acid, and concentrated in a vacuum. The active base thus obtained is said to be twenty-two times more potent than the original alcoholic extract when tested by its power to accelerate fermentation.

A method for the preparation of alimentary products containing vitamin *B* is described as follows: [17a] Wheat bran, or the husk of other edible seeds, is dried at 38° C. under reduced pressure and ground; the powder may be used as such or an aqueous infusion may be prepared from it which can be mixed with gelatin, etc., and formed into tablets — or it may be evaporated under reduced pressure to produce a dry powder. Filtration of the infusion is optional.

Water-soluble *B* also is prepared from wheat germ. This material is treated by McClendon's process in the same manner as in making a concentrate of fat-soluble *A* from green leaves (See p. 111) up to the stage of pressing out the extract, except that 80 per cent alcohol is used. The press-cake is ground in a mill and extracted again in the

[16] A special form of fuller's earth.

[17] **Frankel** and **Schwarz**, Bioch. Zeitsch., 1920, 112, 203.

[17a] *"Forget-me-not-Flours" Ltd., and R. Hutchinson.* British Patent 161,238, Dec. 23, 1919; J. S. C. I. June, 1921, 406A.

same manner. The press juices are concentrated in a vacuum pan to about one-tenth of the original volume or until a precipitate starts to form. An equal volume of water is now added and hydrochloric acid is introduced slowly and with stirring until a bulky and sticky precipitate collects. This is filtered off and the precipitate washed with distilled water and the washings are added to the filtrate. The precipitate is dried and extracted with benzol in an extraction apparatus and the benzol is evaporated. The residue contains some of the wheat oil. The filtrate is brought to pH = 4 to 5 and is fermented until the reducing sugar is lowered to about 1 per cent of the original value. The yeast is filtered off and added to the next batch of wheat germ and the filtrate is evaporated to dryness. In both the filtrate and the benzol extract, water soluble *B* is in quite concentrated form and there is practically no loss except the portion absorbed by the yeast and which would be recovered in the next batch. A very small dose of either the lipoid or water-soluble fraction will cure a pigeon of polyneuritis. The lipoid fraction will revive the hypodynamic heart of a turtle, but the water-soluble fraction is not efficacious. Both fractions stimulate growth.[17b]

In view of the rather extensive use of yeast as a source of *B* the following process for improving the taste of yeast and rendering it more digestible, is of interest.[17c] The yeast may first be treated with a solution containing ammonium or sodium carbonate or borax and washed to remove the bitter flavor, and disintegrated in any known way. It is then treated with hydrogen at a pressure of 100 to 200 atmospheres and a temperature of 100° to 130° C. The liquid thus obtained may be used directly, mixed with fats or oils to form an artificial milk, or dialysed by electro-osmosis or otherwise to remove salts. The action is facilitated by the presence of small quantities of sodium chloride, or organic acids such as formic, acetic, tartaric, or citric acid. The yeast may also be first reduced to a dry powder and treated with hydrogen in this state. Catalysts such as nickel or palladium may be employed in masses, but not as powder. Higher temperatures and lower pressures may be employed, in which case the yeast must be washed after treatments.

Vitamin *C* as found in fruit juices appears to be very easily concentrated. **Harden** and **Zilva**[18] prepared an active antiscorbutic from lemon juice by precipitating the organic acids with calcium

[17b] J. Biol. Chem. 1921. 411. Alcohol of 70% strength containing 0.3% hydrochloric acid and 96% alcohol likewise acidified were used by **Jansen** (Physiol Abs. 5, 361) to extract rice bran. The extracts were applied to washed white rice and dried. The product was fed to cocks and doves. The latter proved slightly more sensitive than cocks to lack of antineuritic vitamin. The methods of extraction gave the same result, the vitamin being quantitatively extracted.

[17c] Illustrated Official Journal, Mar. 9, 1921; **Plauson and Vielle.**

[18] **Harden and Zilva,** Bioch. J. 12, 259.

carbonate and evaporating the filtered juice in vacuo at 30° C., and **Harden and Robinson**[19] report that fruit juices dried in vacuo at low temperatures retain a considerable fraction of their original antiscorbutic potency even after the lapse of two years.[20] **Bassett-Smith**[21] has devised the following process by which antiscorbutic lozenges may be prepared from lemon-juice: Juice of fresh lemons is filtered through muslin, then is passed through filter paper under

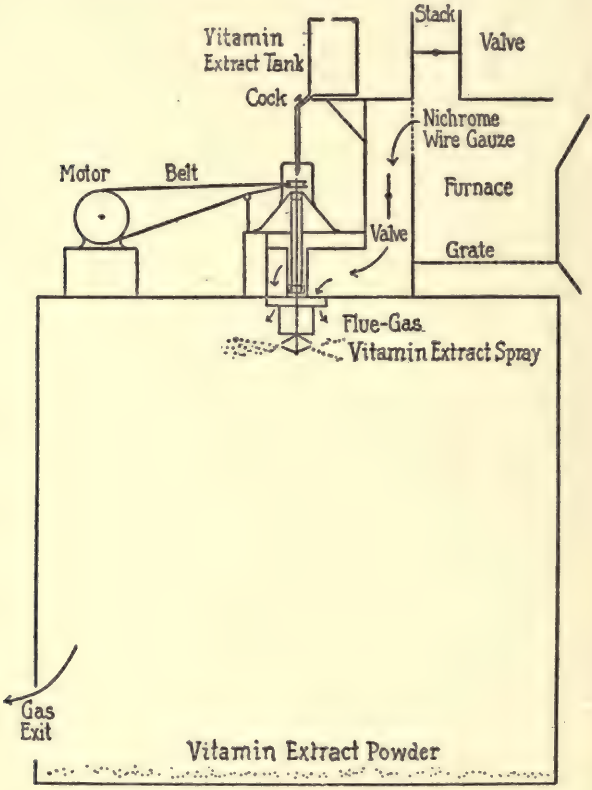

Fig. 5.—McClendon's Drier

reduced pressure. The filtrate is evaporated in vacuo over sulphuric acid at a temperature of 13.5° to 15.5° C. The residue is a non-crystalline syrup, which is worked into as stiff a paste as possible

[19] **Harden and Robinson,** *Ibid.* 14, 171, 1920.

[20] See also **McClendon, Bowers and Sedgwick,** Proc. Soc. Biol. Chem. J. Biol. Chem. 46, ix, 1921; **Givens and Macy,** *Ibid.* xi.

[21] **Bassett-Smith,** Lancet, 1920, ii, 1102.

by incorporation with a dry mixture of anhydrous lactose 97 parts and gum tragacanth 3 parts. The paste is divided into portions, each of which contains the juice of one-half lemon and is equivalent

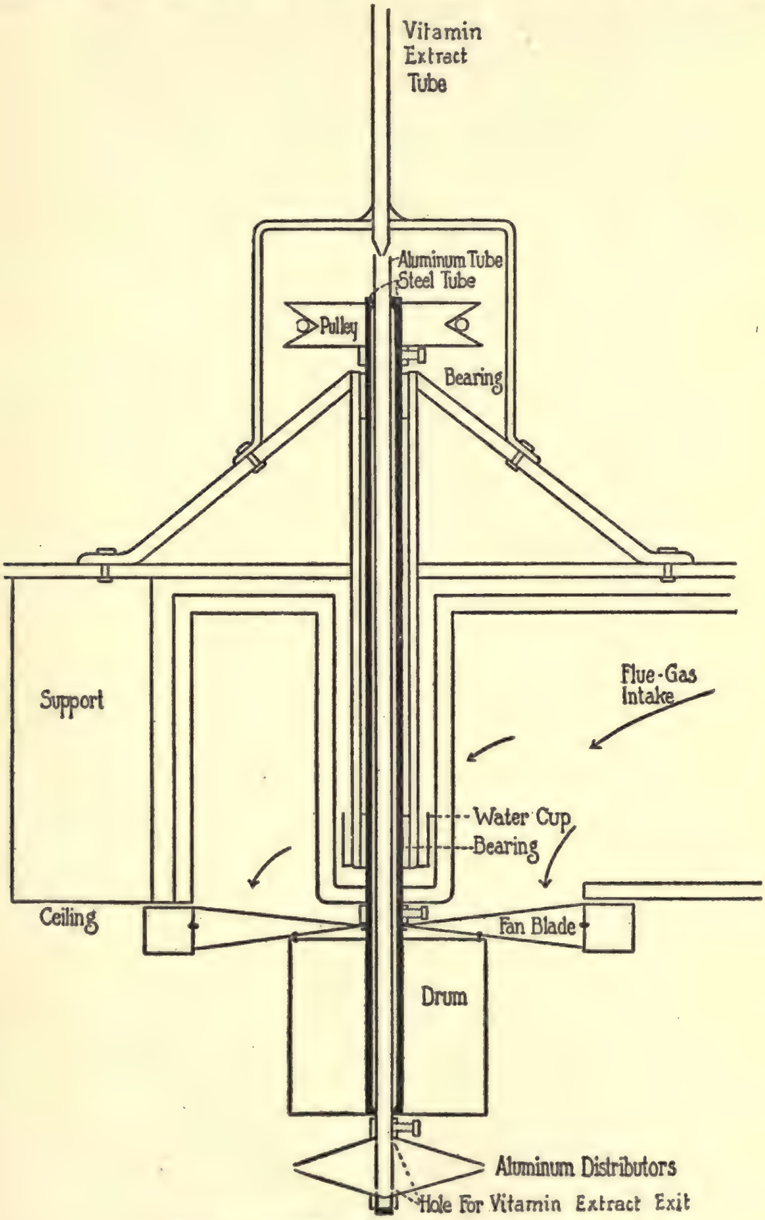

FIG 6.—Details of McClendon's Drier

to 24 cc. of the juice — the dose for an adult. Each portion is rolled, faced with the dry mixture of lactose and gum, and pressed into a lozenge. Five days are required for the entire process of manufacture. The lozenges readily dissolve in water containing a small amount of sodium bicarbonate. These lozenges have been found to retain their efficacy for at least three months at ordinary temperatures. At 37° C. the material darkened gradually, and the vitamin became slightly deteriorated.

Water-soluble *C* is extracted by McClendon from fruits or tomatoes. If oranges are used they are pressed and the juice is run into a vessel previously filled with carbon dioxide so as to exclude the oxygen of the air. Baker's yeast is added and the receptacle is tightly covered.

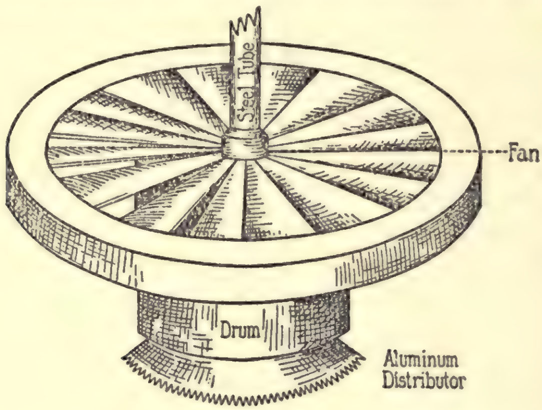

Fig. 7

Fermentation is allowed to proceed at room temperature until the reducing sugar is lowered to about 1 per cent of the original value which requires about 24 to 48 hours. The juice is then filtered with the exclusion of air and is condensed by spraying (in the apparatus described below) in the absence of oxygen, until the volume is reduced to about one-twentieth of the original volume. It is then thrown into four volumes of 95 per cent alcohol and the precipitate thus formed is filtered off and the filtrate sprayed and reduced to dryness with the same precautions. The temperature of drying is about 80° C. 5 to 10 gms. of concentrate *C* were found to be sufficient to prevent scurvy in guinea pigs.[21a]

McClendon and **Dick**[22] have devised a special apparatus for the drying of orange juice and milk on a commercial scale, in which the

[21a] **McClendon,** J. Biol. Chem. 1921, 411.

[22] **McClendon and Dick,** Proc. Soc. Biol. Chem. J. Biol. Chem. 46, x, 1921.

liquid is sprayed into a hot-air chamber (55° to 70° C.). The time of exposure is only one minute, and the antiscorbutic properties of the product are not significantly impaired by the process.

An apparatus used by **McClendon**[22a] for drying extracts of vitamins is shown in Figure 5. A drying chamber supports a charcoal heater furnishing hot flue gas as the drying agent. A tank above the drying chamber contains the vitamin extract which is fed down into the drying chamber through a tube which delivers the liquid to a pair of disc distributors of aluminum. These distributors have saw tooth edges and on rapid revolution of the discs the serrated edges serve to throw off the liquid as a fine spray into the drying chamber. A fan propels the flue gas downwardly across the path of travel of the spray and causes rapid drying of the solid constituents. The dried material in the form of a powder settles to the bottom of the drying chamber and waste flue gas escapes by a vent in the side of the chamber. Figures 6 and 7 show details of the sprayer and fan. Combustion in the furnace must be such as to consume the oxygen and yield some carbon monoxide so that oxidizing conditions will not prevail in the drying chamber.[22b]

Givens and **Macy**[23] have reported favorably upon the antiscorbutic properties of fruit juices dried by the **Merrell-Soule** process.[24] Such juices are said to retain their potency for from 14 to 20 months at least.

Dubin[25] has given the name *Vitaphos* to a preparation made from corn, autolyzed yeast, and orange juice, and said to contain the antineuritic, antiscorbutic and antirachitic vitamins, in stable, active form. A tentative analysis shows 10 per cent calcium oxide, 15 per cent phosphorus (mostly organic), 3 per cent nitrogen and 2 per cent fat. Experiments first with pigeons, guinea pigs, and eventually with children gave results indicating the product possessed marked growth-promoting properties and both preventive and curative properties as regards polyneuritis and scurvy. Cases of rickets treated with

[22a] **McClendon**, J. Biol. Chem. 47, 1921, 415.

[22b] As the operation proceeds, the ceiling of the drying chamber gradually gets hotter and if the operation is not stopped in a short time some of the concentrate is burned on the ceiling. This may be obviated, according to a private communication from Prof. McClendon, by making the ceiling in the form of a shallow metal pan and filling this with water. The authors desire to acknowledge the courtesy of Prof. J. F. McClendon and the Journal of Biological Chemistry in granting permission to use the illustrations relating to the McClendon drier.

[23] *l. c.* xi.

[24] See p. 287.

[25] **Dubin**, Science 51, 71, 1920.

"Vitaphos" showed marked improvements and considerable gain in weight.

An interesting preparation has recently been put on the market by **Page & Shaw,** the well-known candy makers. This is a palatable milk chocolate in which are incorporated concentrated extracts of the three vitamins. Exact information as to the materials used is not at present available, but the manufacturers claim that the process of preparation is such as to preserve the vitamin potency to a high degree, and that it is possible to blend the three vitamins in any desired proportions so as to produce either a balanced ration of the three, or one in which any particular vitamin predominates according to individual need. Clinical tests as to the effects of this chocolate on children and adults are still in progress, but preliminary experiments have given very satisfactory results, especially with respect to vitamin *B*.

Another product on the market, reported to consist of a mixture of chocolate and yeast, is known as Chocolate Yeast or C-Y. The product is in wafer form and has a pronounced chocolate flavor without any odor or taste of yeast. Each wafer is said to contain approximately the same percentage of yeast as is present in a compressed yeast cake. An analysis from the Schwarz Laboratories [26] showed:

Dried Yeast	10.50%
Fat	38.74%
Cane Sugar	31.66%
Cocoa Butter	19.10%

As there is some objection to feeding vitamins in the form of a confection, **Dass** [27] has incorporated yeast and peanut butter to make a desirable food product. The predominating flavor of peanut butter entirely conceals the taste of yeast. The product is stable over a long period and appears to be a very satisfactory way to administer yeast. Dass also has added vitamins from other sources to peanut butter without impairing the flavor of the latter. Yeast decreases the tendency of peanut butter to cling to the roof of the mouth and in this way improves the palatability of the butter.

Hawk, Smith and **Bergheim** emphasize the value of yeast as an addition to food products. They found bread containing 10 per cent of the total protein in the form of milk proteins to be inadequate for the normal growth of white rats. The addition of yeast powder gave a more efficient ration.[27a]

[26] Data furnished through courtesy of Mr. W. B. McLaughlin, President, Chocolate Yeast Co.

[27] Ellis Foster Co., Montclair, N. J.

[27a] Am. J. Physiol. 56, 33, 1921.

A concentrate known as Susto has been examined by **Hawk** and the formula modified by the addition of calcium compounds and dried compressed yeast. The dietetic tests made by Hawk on Susto were favorable.

A vitamin extract for therapeutic administration is put forth by Parke, Davis & Co., under the name of Metagen. The product is of a dark green color with an odor recalling hay and contains water-soluble and fat-soluble vitamins. Metagen is offered for use in deficiency diseases — beriberi, scurvy, marasmus and malnutritions; as a supplemental therapeutic agent in rickets and pellagra; as an adjuvant in ill-defined disorders of nutrition; in convalescence from infectious and other debilitating diseases, and in the nutritional treatment of wasting diseases, anemia and other dyscracias.

The usual dose of Metagen for an infant is half the contents of a five-grain capsule, mixed with a small quantity of water, which is repeated two to four times daily, between feedings. In the cases of older children, three to ten years of age, one capsule should be given three times daily, before meals; for children of ten to sixteen years the number of capsules should be increased to four. Should difficulty be experienced in administering the capsules to children, the contents may be removed and mixed with a small portion of food, provided it is not hot. The adult dose is two capsules two or three times daily, before or after meals.[28]

The suggestion comes from **McClendon**[29] to use the peel of citrus fruits to make a vitamin tablet. The procedure is to grind orange peelings in a meat chopper, then dry and grind in a coffee mill. The powder is made into tablets by the addition of dehydrated orange juice serving as a binder. Such tablets contain vitamins *A, B,* and *C*. Similarly ground spinach may be made into tablets with orange juice.

[28] Various preparations of vitamins combined with certain tonic drugs are now being introduced. Among these may be mentioned "Vitamon," a compound of the three vitamins with small quantities of nux vomica and calcium glycerophosphates. No data is at hand with regard to the theropeutic value of this in human malnutrition.

Drummond (Am. J. Pub. Health 11, 593, 1921) warns against the commercial exploitation of vitamins.

[29] **McClendon,** Science 54, 409, 1921.

CHAPTER VIII

HOW MANY VITAMINS ARE THERE?

The existence of three well-defined vitamins, growth-promoting *A*, antineuritic *B*, and antiscorbutic *C*, is apparently well established. Some evidence has been accumulated which points to the possibility of a fourth, which has usually been included with the antineuritic factor on account of similarity of properties, but which is distinguished from this inasmuch as it is essential for growth but has neither preventive nor curative effect on polyneuritis.

Assuming, in the latter case, that we are dealing with two distinct factors, it will be noted that they show striking uniformity of distribution. Although our present methods of investigation are not sufficiently standardized to make quantitative results entirely convincing, in general it would appear as though foodstuffs which are rich in antineuritic are also rich in growth-promoting power, and those low in antineuritic are also poor in the growth-promoting factor. There are, however, several instances in which this fails to hold true. While a diet containing 88 per cent of unhusked rice, along with casein, salts, and butter-fat, is said to furnish sufficient *B* for normal growth, being comparable to whole wheat in this respect,[1] **Gibson** and **Concepcion**[2] report that an exclusive diet of unhusked rice afforded incomplete protection to pigeons against polyneuritis, as was shown by the degenerative changes in the sciatic nerve. **Voegtlin, Lake** and **Myers**[3] found the endosperm of corn and wheat practically devoid of antinueritic while **Osborne** and **Mendel**[4] assert that wheat endosperm is rich in growth-promoting *B*. According to **Chick** and **Hume**, the onion is poor as a source of antineuritic factor, being about equivalent to fresh meat in this respect,[5] and **Chamberlain, Vedder** and **Williams**[6] report the water extract of onion to be without effect on polyneuritic pigeons; on the other hand,

[1] **McCollum** and **Davis**, J. Biol. Chem. 23, 206, 1915.

[2] **Gibson** and **Concepcion**, Phil. J. Sci. 9, 119, 1914.

[3] **Voegtlin, Lake** and **Myers**, Proc. Soc. Biol. Chem., J. Biol. Chem. 41, x., 1920.

[4] **Osborne** and **Mendel**, J. Biol. Chem. 37, 557, 1919.

[5] **Chick** and **Hume**, Tr. Soc. Med. Hyg. 10, 141, 1916-17.

[6] **Chamberlain, Vedder** and **Williams**, Phil. J. Sci. B7, 45, 1912.

Osborne and **Mendel**[7] found considerable amounts of growth-promoting *B* present. **Chick and Hume** (l. c.) place carrots in the same class with onions and fresh meat as a source of antineuritic factor, and **Davis**[8] found that five grams per day afforded no protection against polyneuritis, yet **Osborne** and **Mendel**[9] and **Suguira** and **Benedict**[10] agree that they are rich in growth-promoting *B*. Very similar evidence is given with regard to the potato. While comparable to carrots in growth-promoting power,[11] it is low in antineuritic factor,[12] although **Davis**[13] secured protection against polyneuritis by administration of eight grams of raw potato per day. Milk is poor in antineuritic substance, according to **Chick** and **Hume**[14] and also **Gibson** and **Concepcion**,[15] but remarkably rich in growth-promoting power, according to **Hopkins.**[16] Although other investigators have not succeeded in attaining normal growth with such extremely small quantities of milk as those used by **Hopkins**, they have demonstrated the presence of at least a fair amount of growth-stimulant. Orange juice is said to contain antineuritic factor in very small amounts,[17] but **Osborne** and **Mendel**[18] found it almost equivalent to milk in growth-promoting power and **Funk** and **Dubin**,[19] using a modification of **Williams'** yeast test, assert that it has about half the value of autolyzed yeast as a growth stimulant.

Several observers have noticed considerable difference in the relative amounts of the same foodstuff which are required to protect against polyneuritis and to induce growth. **Schaumann**[20] ascertained that if a ration of a vitamin-containing foodstuff were adequate to prevent onset of polyneuritis in birds, when this ration was added to a diet of polished rice, loss of weight was prevented. On

[7] **Osborne** and **Mendel**, J. Biol. Chem. 39, 29, 1920.

[8] **Davis**, J. Home Econ. 12, 209, 1920.

[9] **Osborne** and **Mendel**, J. Biol. Chem. 41, 451, 1920.

[10] **Suguira** and **Benedict**, J. Biol. Chem. 36, 171, 191, 1918.

[11] **Osborne** and **Mendel**, J. Biol. Chem. 41, 451, 1920; **McCollum**, **Simmonds** and **Parsons**, *Ib*. 36, 197, 1918.

[12] **Chick** and **Hume**, Med. Res. Com. Report, No. 38, p. 30; **Vedder** and **Clark**, Phil. J. Sci. B. 7, 423; **McCollum** and **Kennedy**, J. Biol. Chem. 24, 491, 1915–16.

[13] **Davis**, J. Home Econ. 12, 209, 1920.

[14] **Chick** and **Hume**, Jour. Roy. Army Med. Corps, 29, 121, 1917.

[15] **Gibson** and **Concepcion**, Phil. J. Sci. 11, 119, 191.

[16] **Hopkins**, J. Physiol. 44, 425, 1912; **Hopkins** and **Neville**, Bioch. J. 7, 97, 1913.

[17] **Harden** and **Zilva**, Bioch. J. 12, 93, 1918.

[18] **Osborne** and **Mendel**, J. Biol. Chem. 42, 465, 1920.

[19] **Funk** and **Dubin**, J. Biol. Chem. 44, 492, 1920.

[20] **Schaumann**, Trans. Soc. Trop. Med. Hyg. 5, 59, 1911.

the other hand, extracts of these foodstuffs prepared by extraction with acid or alcohol, while still sufficient to prevent polyneuritis, were no longer able to prevent loss of weight.

Voegtlin and **Towles** [21] noted that the extracts of autolyzed spinal cord may be antineuritic, yet be unable to re-establish normal metabolism, i. e., restore body weight. **Cooper** came to the conclusion that the substances preventing polyneuritis and maintaining weight were separately distributed in natural foodstuffs, since for certain of these, in which the two appeared to be evenly balanced, such as yeast, ox-heart, and ox-brain, the daily ration required to prevent polyneuritis also maintained the weight of the bird, while in others, such as egg-yolk, barley, lentils, this daily ration had to be increased if loss of weight were to be prevented. **Chick** and **Hume** [22] observed that while three grams of wheat germ every second day prevents polyneuritis, two to three grams daily produced great improvement in body weight, health, and vitality.[23] These results might be interpreted either as due to different requirements of the same vitamin for different metabolic functions or to two vitamins which are present in different amounts. The evidence is not convincing on either side. **Mitchell** (l. c.) remarks: In investigations on the comparative antineuritic properties of foods, the experimental polyneuritis of pigeons is almost invariably induced by a diet of polished rice. Polished rice is defective in several factors beside the antineuritic. It is therefore probable that foods containing comparable amounts of this vitamin may be unequally effective in preventing or curing the polyneuritic symptoms, depending upon the extent to which they supplement polished rice in these other respects. Conversely, foods containing unequal concentrations of the antineuritic principle may have their relative values as sources of the vitamin distorted for the same reason.

Funk [24] noted that inhibition of growth may be caused by a diet containing vitamin, and regarded this as indication that the growth-vitamin is not identical with the anti-beriberi vitamin. This conclusion was not justified, however, since the diet employed was undoubtedly deficient in *A*. Later[25] he attempted to separate a growth-stimulating fraction from yeast by the methods which he employed for separating an antineuritic fraction. He states:

[21] **Voegtlin and Towles,** J. Exp. Pharmocol. 5, 67, 1913.
[22] **Chick and Hume,** Proc. Roy. Soc. 90B, 44.
[23] **Chick and Hume,** Proc. Roy. Soc. 90B, 44.
[24] **Funk,** Z. Physiol. Chem. 88, 352, 1913.
[25] **Funk and Macallum,** J. Biol. Chem. 27, 63, 1916.

The fractionation of yeast with phosphotungstic acid shows that the growth-promoting substance is carried down with the precipitate and a large part of its activity is lost during the fractionation. The instability of this substance when fractionated with silver salts presents greater difficulty than that experienced during the fractionation of the beriberi vitamin. . . . The experimental evidence indicates that considerably larger quantities of vitamins are necessary for stimulating growth than for curing beriberi, and the losses occurring during fractionation are more apparent in the former than in the latter case. However, it must be admitted that while it is uncertain whether these two substances are chemically different, the results obtained do not exclude such a possibility. Lloyd's reagent, as recommended by **Seidell,** has also been used as a precipitant without much success, as rats which have been given the filtrate have also shown increments in growth.

All attempts to distinguish between the two substances by their behavior towards precipitants have proved a failure. While the two seem to be precipitated by the same reagents, the loss of activity in the course of manipulation is so large that no satisfactory conclusions can be reached.[26] **Mitchell**[27] points out that the growth-promoting factor appears to suffer more extensive deterioration during fractionation than does the antineuritic, which may be regarded as an indication of the existence of two substances instead of one, although it can hardly be considered a conclusive argument. **Drummond** remarks that it is naturally very difficult to trace the growth-promoting principle during fractionation, because minute traces of that substance do not always demonstrate their presence by causing appreciable increase in the body weight of the experimental animal, whereas such extracts are often sufficient to cause decided improvement in the condition of a polyneuritic pigeon.[28]

Both the antineuritic and the growth-promoting factors are soluble in water and dilute alcohol. According to **Osborne** and **Mendel**[29] and **Drummond,**[30] the growth-promoting factor of yeast is insoluble in absolute alcohol. The antineuritic, on the other hand, is said to be extracted by strong alcohol from the foodstuffs containing it.[31] While acetone and benzene extracts of fat-free wheat embryo are as efficient as water or alcohol in curing polyneuritis, these solvents are not successful in extracting the growth-promoting *B* from foodstuffs.[32]

[26] See **Funk and Macallum,** l. c.; **Eddy,** J. Biol. Chem. 27, 113, 1916: **Drummond,** Bioch. J. 11, 255, 1917.

[27] **Mitchell,** J. Biol. Chem. 40, 399, 1919.

[28] **Drummond,** Bioch. J. 11, 255, 1911.

[29] **Osborne and Mendel,** J. Biol. Chem. 31, 149, 1917.

[30] **Drummond,** Bioch. J. 11, 261, 1918.

[31] **Eijkman,** Arch. f. path. Anat. 148, 523; 149, 197; **Fraser and Stanton,** Lancet, 1910, ii, 1755; **Funk,** J. Physiol. 43, 395, 1911-12; **Cooper,** J. Hyg. 12, 436, 1913.

[32] **McCollum and Kennedy,** J. Biol. Chem. 24, 491.

These facts would seem to indicate the existence of two factors, but there are sufficient discrepancies in the statements as to solubility, even where there is no question of more than one vitamin being involved, to make us chary of drawing conclusions from this property.[33]

The stability of the two towards acids and alkalies seems on the whole to be very similar. **Williams** and **Seidell**[34] have reported an experiment in which they found that when fuller's earth, which had been activated by contact with autolyzed yeast filtrate, was treated with aqueous alkali it lost its growth-promoting power, whereas its antineuritic efficacy was unchanged. **Emmett** and **McKim**,[35] however, found that even without the alkali treatment, activated fuller's earth had very little effect in promoting the growth of pigeons, being very inferior to the natural foodstuffs in this respect. They conclude that the outer layers of the rice grains contain two vitamins, one of which cures or prevents polyneuritis and the other produces growth, and of these two the yeast vitamin preparation obtained with fuller's earth contains chiefly the curative fraction.

Somewhat more convincing evidence has been obtained from a study of the relative stability of the two towards high temperatures. This has been very carefully investigated by **Emmett** and his coworkers, from whom the accompanying table summarizing the results of earlier investigators has been taken.[36]

[33] **McCollum** and **Davis**, J. Biol. Chem. 23, 229, 1915; **McCollum** and **Simmonds**, *Ibid.* 33, 55, 1918.

[34] **Williams** and **Seidell**, J. Biol. Chem. 26, 432, 1916.

[35] **Emmett** and **McKim**, J. Biol. Chem. 32, 409, 1917.

[36] **Emmett** and **Luros**, J. Biol. Chem. 43, 265, 1920.

POLYNEURITIS (ANTINEURITIC VITAMIN)

Series	Temperature and time of heating	Destruction	Reference
	Section I. Fowl: Pigeon, chicken, and duck.		
A	100° C., moist heat		
1a †	Egg yolk, 4 min.	None apparent	Cooper (1)
2a	" " in presence of dilute alkali	Total	Steenbock (2)
3b †	Buffalo meat, several days	"	Grijns (3)
4b	Beef, 30 min.	None apparent	Holst (4)
5a	Yeast extract, 1 hr.	Slight	Chick and Hume (5)
6a	Wheat embryo, 1 hr.	Very slight	Chick and Hume (5), (6)
7b	Unmilled rice, 3 hrs.	None apparent	Eykman (7)
B	110°, autoclave, 30 min.		
8b	Beef	Appreciable	Holst (4)
C	113°, autoclave, 1 hr.		
9a	Wheat embryo (102–107° for 40 min.) ‡	Slight	Chick and Hume (6)
D	115°, autoclave, 2 hrs.		
10b	Unmilled rice, millet, oats, rye, barley	Total	Eykman (7)
E	120°, 15 pounds pressure, 30 min.		
11b	Beef, eggs	Marked	Vedder (8)
12b	Dried peas, unhulled barley	None apparent	Holst (4)
F	120°, 15 pounds pressure, 1 hr.		
13b	Beef	Total	" "
G	120°, 15 pounds pressure, $1\frac{1}{2}$ hrs.		
14b	Unmilled rice	"	Weill, Mouriquand, and Michel (9)
15b	Barley	"	Weill, and Mouriquand (10)

† a, tested curatively by giving it to polyneuritic fowl; b, tested prophylactically by feeding normal fowl.

‡ Temperature of the substance itself and length of time it remained at this point.

(1) Cooper, E. A., J. Hyg., 1912, xii, 448.
(2) Steenbock, H., J. Biol. Chem. 1917, xxix, p. xxvii.
(3) Grijns, Geneesk, Tijdschr. v. Ned. Ind., 1901, xli, 30.
(4) Holst, H., J. Hyg., 1907, vii, 619.
(5) Chick, H., and Hume, E. M., J. Roy. Army Med. Corps., 1917, xxix, 121.
(6) Chick, H., and Hume, E. M., Proc. Roy. Soc. London, Series B, 1919, xc, 44.
(7) Eykman, C., Arch. Hyg., 1906, lviii, 150.
(8) Vedder, E. B., J. Hyg., 1918, xvii, 1.
(9) Weill, E., Mouriquand, G., and Michel, P., Compt. rend. Soc. biol. 1916, lxxix, 189.
(10) Weill, E., and Mouriquand, G., Compt. rend. Soc. biol., 1915, lxxviii, 649.

POLYNEURITIS (ANTINEURITIC VITAMIN) — *Continued*

Series	Temperature and time of heating	Destruction	Reference
	Section I. Fowl: Pigeon, chicken, and duck. — Concluded		
H	120°, 15 pounds pressure, 2 hrs.		
16b	Unmilled rice, Katjidgo beans, buffalo meat	Total	Grijns (3), Eykman (7)
17b	Horse meat	None apparent	Eykman (7)
18b	Rye, unmilled rice, millet, oats, barley	Total	Holst (4)
I	122°, autoclave, 1 hr.		
19a	Yeast extract and wheat embryo (110–117° for 40 min.) ‡	Appreciable	Chick and Hume (5, 6)
J	122°, autoclave, 2½ hrs.		
20a	Yeast extract and wheat embryo (118–124° for 2 hrs.) ‡	Very marked	Chick and Hume (5, 6)
K	125°, autoclave, 2 hrs.		
21b	Unmilled rice and millet	Total	Eykman (7)
L	135°, autoclave, 2 hrs.		
22b	Unmilled rice, rye, millet, oats, barley	"	Holst (4)
	Section II. Dogs		
M	120–130°, autoclave, 1 to 3 hrs.		
23b	Horse meat	Total	Schaumann (11)
24b	Lean beef in presence of 10 per cent Na_2CO_3	"	Voegtlin and Lake (12)
	Section III. Cats		
N	120°, 15 pounds pressure, 3 hrs.		
25b	Lean beef	Appreciable	Voegtlin and Lake (12)
26b	" " in presence of 10 per cent Na_2CO_3	Total	Voegtlin and Lake (12)

‡ Temperature of the substance itself and length of time it remained at this point.

(3) Grijns, Geneesk, Tijdschr. v. Ned. Ind., 1901, xli, 30.
(4) Holst, H., J. Hyg., 1907, vii, 619.
(5) Chick, H., and Hume, E. M., J. Roy. Army Med. Corps., 1917, xxix, 121.
(6) Chick, H., and Hume, E. M. Proc. Roy. Soc. London, Series B, 1919, xc, 44.
(7) Eykman, C., Arch. Hyg., 1906, lviii, 150.
(11) Schaumann, H., Arch. Schiffs-u. Tropenhyg., 1910, suppl., xiv, 64.
(12) Voegtlin, C., and Lake, G. C., Am. J. Physiol., 1918-19, xlvii, 558.

GROWTH–PROMOTING VITAMIN (WATER–SOLUBLE B)

Series	Temperature and time of heating	Destruction	Reference
	Section I. Rats		
A	90–100° C., dry heat, several hrs.		
1	Liver, heart, kidney, brain	None apparent	**Osborne and Mendel** (13)
B	100°, moist heat		
2	Protein, free milk, 2 min.	None apparent	**Osborne and Mendel** (14)
3	Milk whey, 6 hrs.	" "	**McCollum and Davis** (15)
4	Extract yeast, 30 min.	" "	**Drummond** (16)
5	Extract wheat embryo, in presence of 0.28 per cent NaOH, 1 hr.		**McCollum and Simmonds** (17)
6	Extract yeast, in presence of 5 per cent NaOH, 5 hrs.	Marked	**Drummond** (16)
7	Soy beans, navy beans, cabbage, 40 to 120 min.	None apparent	**Daniels and McClurg** (18)
8	Soy beans (120 min.), navy beans (90 min.), cabbage (45 min.) in presence of 5 per cent $NaHCO_3$.	" "	**Daniels and McClurg** (18)
9	Carrots †	" "	**Denton and Kohman** (19)
10	Yeast, 0.1 N NaOH for 21.5 hrs. in cold. 2 hrs. heating	" "	**Osborne, Wakeman and Ferry** (20)
C	105°, dry heat, several hrs.		
11	Meat powder (lean beef)	" "	**Osborne and Mendel** (21)
12	Beef extract	" "	**Osborne and Mendel** (21)
13	Compressed yeast	" "	**Hawk, Fishback, and Bergeim** (22)

† Placed in cans, then immersed in water, and heated at 100° for 2 hrs.

(13) **Osborne, T. B., and Mendel, L. B.,** J. Biol. Chem. 1918, xxxiv, 17.
(14) **Osborne, T. B., and Mendel, L. B.,** Carnegie Inst. of Washington, Pub. 156, pts. i and ii, 1911.
(15) **McCollum, E. V., and Davis, M.,** J. Biol. Chem. 1915, xxiii, 247.
(16) **Drummond, J. C.,** Biochem. J. 1917, xi, 255.
(17) **McCollum, E. V., and Simmonds, N.,** J. Biol. Chem. 1918, xxxiii, 55.
(18) **Daniels, A. L. and McClurg, N. I.,** J. Biol. Chem. 1919, xxxvii, 201.
(19) **Denton, M. C., and Kohman, E.,** J. Biol. Chem. 1918, xxxvi, 249.
(20) **Osborne, T. B. Wakeman, A., and Ferry, E. L.,** J. Biol.Chem. 1919, xxxix, 35.
(21) **Osborne, T. B., and Mendel, L. B.,** J. Biol. Chem. 1917, xxxii, 309.
(22) **Hawk, P. B., Fishback, H. R., and Bergeim, O.,** Am. J. Physiol. xlviii 211.

GROWTH-PROMOTING VITAMIN (WATER-SOLUBLE B) — *Continued*

Series	Temperature and time of heating	Destruction	Reference
	Section I. Rats (Continued)		
D	120°, 15 pounds pressure, 20 min.		
14	Navy beans, cabbage	None apparent	**Daniels** and **McClurg** (17)
15	Extract, navy beans	" "	**Daniels** and **McClurg** (17)
E	120°, 15 pounds pressure, 30 min.		
16	Extract yeast	Marked	**Drummond** (15)
17	Soy bean flour	None apparent	**Cohen** and **Mendel** (23)
18	Extract, navy and soy beans, in presence of 0.1 N NaOH	" "	**Daniels** and **McClurg** (18)
F	120°, 15 pounds pressure, 40 min.		
19	Navy beans	None apparent	**McCollum** and **Simmonds** (17)
20	Soy beans	" "	**Daniels** and **McClurg** (18)
21	Extract, soy beans	" "	**Daniels** and **McClurg** (18)
G	120°, 15 pounds pressure, 1 hr.		
22	Wheat embryo, milk whey	" "	**McCollum** and **Davis** (15)
23	Extract, navy beans, in presence of 0.1 N NaOH	" "	**Daniels** and **McClurg** (17)
H	120°, 15 pounds pressure, $1\frac{1}{4}$ hrs.		
24	Navy bean	" "	**McCollum**, **Simmonds** and **Pitz** (24)
I	120°, 15 pounds pressure, 3 hrs.		
25	Lean beef, in presence of 10 per cent Na_2CO_3	None apparent	**Voegtlin** and **Lake** (12)
	Section II. Yeast cell		
J	120°, 15 pounds pressure, 30 min.		
26	Yeast extract	Slight	**Williams** (25)

(12) **Voegtlin, C.**, and **Lake, G. C.**, Am. J. Physiol. 1918–19, xlvii, 558.
(15) **McCollum, E. V.**, and **Davis, M.**, J. Biol. Chem. 1915, xxiii, 247.
(16) **Drummond, J. C.**, Biochem. J., 1917, xi, 255.
(17) **McCollum, E. V.**, and **Simmonds, N.**, J. Biol. Chem. 1918, xxxiii, 55.
(18) **Daniels, A. L.**, and **McClurg, N. I.**, J. Biol. Chem. 1919, xxxvii, 201.
(22) **Cohen, B.**, and **Mendel, L. B.**, J. Biol. Chem. 1918, xxxv, 425.
(23) **McCollum, E. V.**, **Simmonds, N.**, and **Pitz, W.**, J. Biol. Chem. 1917, xxix, 521.
(25) **Williams, R. J.**, J. Biol. Chem., 1919, xxxviii, 465.

Emmett and **Luros** (l. c.) compare the effect of the same foodstuff on polyneuritis in pigeons and on the rate of growth of rats, using unmilled rice as the exclusive diet for pigeons [37] and the basal diet, supplemented with lactalbumin salt mixture, butter-fat, and

[37] See **Gibson** and **Concepcion**, Phil. J. Sci. 9, 119, 1914.

lard for rats. They obtained evidence that the antineuritic vitamin (tested on pigeons) in unmilled rice is stable to heat at 120° C. and 15 pounds pressure for one hour. It is partially altered by heating in the air oven at 120° C. for two hours, and totally destroyed at 120° C. and 15 pounds pressure in two and six hours. The vitamin in extracts is more easily altered by heat. The water-soluble *B* vitamin (tested on rats) in unmilled rice appears to be stable to heat at these same temperatures, that is, it is not distinctly or totally broken down. Whether this vitamin was slightly destroyed could not be definitely ascertained due to the lack of quantitative methods.

Emmett and **Stockholm**[38] endeavored to obtain further evidence on this point by using the **Williams'** method as a test for the growth-promoting *B*. Tests were made with unmilled rice, unheated and heated in the autoclave for 1, 2 and 6 hours respectively at 120° C. and 15 pounds pressure. They found no indication that the factor which stimulated the growth of yeast cells was altered in the least by heating. When the heated rice was tested on polyneuritic pigeons it was found to be entirely lacking in antineuritic power. Normal rats fed on this heated rice gained in weight, although the gain on the rice which had been autoclaved six hours was only moderate. It is noteworthy, however, that rats which have been brought to a low nutritive plane through lack of *B* persistently lost weight on extracts of heated rice (autoclaved for two hours), while responding promptly to dosage with extract of unheated rice. This suggests the possibility that the yeast-stimulant is not identical with growth-promoting *B*.

Much work remains to be done before this point can be definitely settled. Since, however, it would seem wise to keep in mind the possibility of the existence of two vitamins of the *B* type, we have endeavored, so far as possible, to indicate whether it is the antineuritic or the growth-promoting power of *B* which is taken into consideration.

From autolyzed yeast **Funk** and **Dubin**[39] were able to separate one substance active for yeast and another which was active for rats and pigeons, showing that yeast requires for growth a different substance than that needed by animals. They regard the yeast-active substance as a new vitamin or a cleavage product of vitamin *B* and provisionally call this substance Vitamin *D*. An almost quantitative separation of this vitamin from autolyzed yeast may be made by two successive shakings with 100 grams of fuller's earth or of norit, per litre. The fuller's earth and norit are decomposed by baryta and

[38] Emmett and Stockholm, J. Biol. Chem. 43, 287, 1920.

[39] J. Biol. Chem. 48, 437, 1921.

glacial acetic acid respectively, according to the method of Seidell and of Eddy and co-workers. Norit extracted with baryta does not yield an active substance. Vitamin *D*, according to Funk and Dubin, appears to be a definite and specific substance stimulating the growth of yeast. Although vitamin *D* has been obtained from vitamin *B*, as far as animal experiments have shown, the reverse is not true. If therefore most animal tests conducted up to the present time were carried out, with a mixture of vitamins *B* and *D* it will be desirable to repeat these tests with the separate vitamins.[40]

Considerable work has been done by **Freedman**[41] and others in ascertaining the identity of the substance stimulating the growth of yeast cells and its relation to the growth of bacteria. Working with a strain of hemolytic streptococci and pure yeast cultures, Freedman was able to demonstrate that beef and beef heart infusions, peptone and autolyzed yeast contained substances that are equally active with respect to the growth of these organisms. Certain specially prepared proteins such as casein, albumin, zein, fibrin, gelatin, edestin and orycinin apparently contain these substances which are active for bacteria. Freedman believes these active substances to be similar to, or identical with the yeast growth-promoting vitamin *D*.[42]

Pacini and **Russell**[43] apparently demonstrated the presence of a growth-promoting substance in extracts of cultures of *Bacillus typhosus*. **Thjötta** also showed that a favorable influence is exerted on *Bacillus influenzae* by the addition of sterile bacterial extracts to the broth in which the organism is planted.[44] On the other hand, **Cooper**[45] has found that extracts of *Bacillus coli* have no effect in relieving polyneuritis in pigeons. **Damon**[46] has endeavored to throw further light on the subject by feeding to rats the products of the growth of *Bacillus paratyphosus B, Bacillus coli* and *Bacillus subtilis*. The administration of the bacteria was carried out in the following way: The organisms were grown in flasks containing 100 cc. of the medium, then killed by autoclaving at 120° for fifteen minutes — a procedure that would not affect the vitamin content as has been demonstrated by **McCollum, Simmonds,** and **Pitz.**[47] The culture

[40] See also **Emmett** and **Stockholm**, J. Biol. Chem. **43**, 287, 1920.

The anti-beriberi vitamin and the separation of vitamin *D* are discussed by **Funk** (J. Ind. Eng. Chem. 1921, 1110).

[41] Am. Food Jour. Oct. 1921.

[42] In yeast there is present some constituent, soluble in 70 to 95 per cent alcohol and still more soluble in water, which, when added in small amounts to a medium in which yeast is grown, will increase the invertase activity of yeast to a remarkable extent (**Miller**, J. Biol. Chem. **48**, 329, 1921). This substance, however, is not identical with the growth stimulant.

[43] J. Biol. Chem. **34**, 43, 1918.

[44] J. Exp. Med. **33**, 763, 1921.

[45] J. Hyg. **14**, 20, 1914.

[46] J. Biol. Chem. **48**, 379, 1921.

[47] J. Biol. Chem. **29**, 521, 1917.

was then concentrated by evaporation on the steam bath to about 15 to 20 cc. and finally the organisms were taken up on starch by desiccation in a shallow pan at a reduced pressure. This starch bearing the bacteria was then used to replace an equivalent amount in the basal ration and the effect of bacteria noted by observing the trend of the weight curve. No indication was found that water-soluble *B* was formed by the organisms examined.

Suguira and **Benedict** [48] have suggested the possibility that milk contains still another accessory substance which is lacking in yeast. They base this hypothesis on their observation that a diet of bananas, casein, and yeast is entirely satisfactory for growth and reproduction of rats but is not sufficient to permit of rearing the young, while the addition of 10 cc. of cows' milk to the diet of the mother resulted in normal growth of the sucklings. The necessary addition could be obtained from " protein-free milk " [49] but not from purified lactose or the inorganic constituents of milk.

[48] **Suguira** and **Benedict**, J. Biol. Chem. 40, 449, 1919.
[49] See pp. 9, 10.

CHAPTER IX

RÔLE IN NUTRITION

In considering the part played by any factor in the nutrition of an animal it is necessary to distinguish between what is adequate for maintenance and what is required for optimum growth. This has been repeatedly emphasized by students of nutrition, especially in connection with protein metabolism.[1]

With regard to the vitamins it seems clear that both *A* and *B* are required for maintenance and growth, although the relative amount necessary and the precise part played in the physiological processes are still unsolved problems. The rôle of *C*, apart from its antiscurvy effect, is also questionable.[1a]

The early experimenters believed that maintenance could be secured on diets free from *A*, but this has since been shown to be a misconception.[2] **McCollum** and **Kennedy** say: [3]

> Judging from the appearance of serious nutritional disturbances ending in death, which result from a shortage of the fat-soluble *A*, and the emaciation, weakness, and death which follow restriction to a diet inadequate in its content

[1] **McCollum,** Am. J. Physiol. 29, 235, 1911; **Osborne, Mendel and Ferry,** Zeitschr. Physiol. Chem. 1912, 80, 307; **Osborne** and **Mendel,** Carn, Pub. 156, 1911, Sci. 34, 722; J. Biol. Chem. 12, 473; 13, 232; 15, 311; 17, 325; 23, 439.

[1a] **Ganassini** and **Mancini** (Bull. gén. Thérap. 171, 125 1920; Endocrinology 4, 457) express the opinion that the physiological rôle of vitamins is to regulate trophic exchanges in synergic connection with the trophic regulatory function of the products of the glands of internal secretion.

Pigeons on a diet devoid of vitamin *B* exhibited degeneration of the tubules and hyperplasia of the interstitial tissue in the testis. (**Novaro,** Physiol. Abs. 5, 473).

Many experiments on pigeons carried out by **Green** (Physiol. Abs. 5, 305) show that the daily demand for the vitamin *B* is not definite, but varies with the exogenous metabolism. There appears to be no reason to think that vitamin consumption is related to carbohydrate metabolism any more than that of fat or protein. Vitamin *B* is also required for tissue building. The term "vitamin index" is used to denote the ratio of vitamin absorbed in digestion to the energy value of the digested food.

[2] **Osborne** and **Mendel,** J. Biol. Chem. 16, 428, 1913-14; **McCollum** and **Davis,** 15, 168, 1913.

[3] **McCollum** and **Kennedy,** J. Biol. Chem. 24, 500, 1915-16.

of the water-soluble *B*, it seems certain that both these classes of unknown dietary constituents are essential for maintenance as well as for growth. . . . The fat-soluble *A* appears to be dispensable when maintenance alone is involved, for a somewhat longer period than is the factor *B*. This is inferred from the fact that pigeons can be brought into the polyneuritic state by feeding a diet free from both the essential factors *A* and *B*. and can be completely cured and maintained in a normal condition for at least 35 days on the same diet which brought on the disease, plus the water extract of a foodstuff (rolled oats) on which rats cannot grow without the addition of butter-fat, but on which they do grow when the latter is added.

Emmett and **Allen**[4] report that tadpoles require both *A* and *B* for normal growth and development, lack of *B* being possibly more apparent than lack of *A*.

McCollum and **Simmonds**[5] conducted a series of experiments on rats designed to show a quantitative difference in the vitamin requirements (*A* and *B*) for maintenance and growth. Their basal ration consisted of 18 per cent casein, 76.3 per cent dextrin, 3.7 per cent salt mixture and 2 per cent of agar-agar, to which was added varying amounts of butter-fat as source of *A* and of wheat germ as source of *B*. They state:

Results indicate that there is no low plane of intake of either of these substances which can be said to maintain an animal without loss of vitality. When the minimum amount necessary for the prevention of loss of weight is approached the life of the animal is jeopardized if the diet is continued. A quantity of either the fat-soluble *A* or the water-soluble *B* which may be just sufficient when all other dietary factors are of satisfactory quality, will not, when a second factor is less well constituted, induce well-being in the same degree as when a more generous supply is furnished. The animal can tolerate being limited to a very low intake of either the dietary *A* or *B* much better with an otherwise excellent diet than when it is less well constituted.

It is to be noted that **Von Groer**[6] has succeeded in obtaining practically normal growth in two babies for the first six months of life on a diet almost devoid of fat[6a] and correspondingly poor in *A*. The children were not in a satisfactory nutritive condition, however, as was shown by their susceptibility to illness. In any case the experiment cannot be considered an argument against the indispensability of *A* since the centrifuged milk which formed a large portion of the diet was not entirely free from *A*, and the experimental period repre-

[4] **Emmett** and **Allen,** J. Biol. Chem. 38, 325, 1919.

[5] **McCollum** and **Simmonds,** J. Biol. Chem. 32, 185, 1917.

[6] **Von Groer,** Bioch. Z. 97, 311, 1920.

[6a] See **Mackay,** Biochem. J. 15, 19, 1921, and **Tozer,** *Ib.* 28, on the effect on kittens of a diet deficient in fat.

sented only a very small fraction of the life span of a human being. There are frequent similar instances in animal experimentation of periods of growth on a diet which subsequently proved insufficient to maintain life.[7]

It is generally believed that animals have the power of storing up a certain amount of vitamin in the body from which they can draw in case of need, although this store is soon depleted when the food is entirely lacking in vitamins.[8] It is difficult to get direct evidence on this point, since it is impossible to be sure that a supposedly vitamin-free food does not contain traces of the essentials which may be sufficient for the particular individual or species under consideration for a certain length of time, although long continuance on a subminimal supply eventually results in nutritional failure. **Chick** and **Hume**[9] argued from the very close approximation between the daily ration of wheat germ or yeast[10] required to prevent the onset of polyneuritis and the dose necessary to cure a bird in the acute condition, that a considerable store of *B* is available somewhere in the animal body which may be drawn upon to maintain the metabolism of the nerve tissue. This store becomes suddenly exhausted, but can be temporarily restored by the administration of a small quantity of the missing factor in the food. **McCarrison**[11] judged from their atrophy, out of all proportion to other tissues during vitamin starvation, that the thymus, the testicles, the ovary, and the spleen provide a reserve of accessory food factors for use on occasions of metabolic stress. This reserve is, however, rapidly exhausted. **Osborne** and **Mendel** were formerly inclined to believe that young animals maintained a store of *A* in their cells on which they could grow for a time, but later[12] experience has not led to definite confirmation of this hypothesis. With regard to *C*, the work of **Parsons**,[13] referred to below, would seem to indicate that this is more probably synthesized, by certain animals at any rate, rather than stored in the tissue to any considerable extent.[14]

Food with a low vitamin content fed to cows has a deleterious effect on the offspring and according to the observations of **Hughes, Fitch**

[7] **McCollum** and **Davis**, J. Biol. Chem. 23, 233, 1915; **Osborne** and **Mendel**, *Ibid.* 45, 148, 1920.

[8] **Lumiere**, Par. Med. 10, 474, 1920, dissents from this view.

[9] **Chick** and **Hume**, Proc. Roy. Soc. 90B, 55.

[10] **Cooper**, J. Hyg. 12, 436, 1913; Bioch. J. 8, 250, 1914.

[11] **McCarrison**, Ind. J. Med. Res. 6, 275, 550, 1917.

[12] **Osborne** and **Mendel**, J. Biol. Chem. 15, 319; 16, 143.

[13] **Parsons**, J. Biol. Chem. 44, 587, 1920.

[14] **Osborne** and **Mendel**, J. Biol. Chem. 44, 148, 1920.

and **Cave**,[14a] in some cases calves nursed on substances deficient in vitamins become blind. In one test four calves were under observation.

Two were from cows which had received a feed low in vitamin during the entire gestation period; the other two were from cows which had received normal feed. During the first week the two calves from the experimental cows received their mothers' milk. At this time one of these cows died and her calf was then given the other experimental cow's milk. The two calves from the cows receiving normal feed were fed on herd milk exclusively. All four calves wore muzzles so they could get no other feed. All the calves seemed to be normal for the first five weeks, at which time one of the calves receiving the experimental milk became blind. It died when it was forty-two days old, showing nervous symptoms very much like an animal with beriberi. The other experimental calf became blind when seventy-eight days old and died nineteen days later with symptoms like the first. A calf from a cow receiving normal feed was placed on the experimental milk at the time the first calf died. It did not become blind but died at the end of nineteen weeks. The two calves receiving the herd milk were still normal after a period of eight months. The vitamin content of the feed received by the experimental cows and the milk they produced has been tested by experimental animals. The milk is much lower in its water-soluble and fat-soluble vitamin content than the herd milk, but is equal to the herd milk in its antiscorbutic vitamin content. The experiment shows that although a cow receiving a feed low in vitamins may give a fairly abundant supply of milk, it is of such poor quality that it is not an adequate food for her young.[14b]

The question of how far the results obtained from observation on one species of animal can be used in drawing conclusions as to the requirements of another species has already been considered briefly.[15] Unquestionably there are certain differences between the different species in this respect. **McCollum** and **Kennedy**[16] assert that the maintenance requirement of pigeons for *A* is less than the growth requirement of the rat. On the other hand, **Steenbock, Kent** and **Gross**[17] found that the requirements of birds for *B* were far greater than those of the rat.

[14a] Am. Food Jour., Oct., 1921.

[14b] **Hume** (Biochem. J. **15**, 163, 1921) carried out an investigation on the antiscorbutic value of full cream sweetened condensed milk. Full cream milk was heated in a vessel with continuous inflow and outflow at a temperature of 80° C. The milk remained at this temperature for about three and one-half minutes. The sugar was next added, and at this point there was access of air. The milk next passed to a vacuum condenser where it boiled at about 50° for 3 hours and was drawn off after it passed a certain viscosity test. It was then cooled in bulk, and here again it was exposed to the air. The experiments indicated that there is no loss of antiscorbutic potency in this condensed milk. The result is attributed to the fact that the milk is concentrated in vacuo.

[15] Chapter 2.

[16] **McCollum** and **Kennedy**, J. Biol. Chem. 24, 497, 1915-16.

[17] **Steenbock, Kent,** and **Gross**, J. Biol. Chem. 35, 61, 1918.

The most striking difference between the species would seem to lie in the antiscorbutic requirement. The ease and rapidity with which scorbutic symptoms can be produced in a guinea pig by a diet deficient in *C* shows remarkable susceptibility to this lack. Man and monkeys appear to be almost equally dependent on this factor, while rats can be grown and maintained over long periods without any appearance of scorbutic symptoms on diets which contain too little antiscorbutic, if any, to have any effect in delaying the onset of scurvy in the guinea pig, and the requirement of the prairie dog apparently resembles that of the rat in this respect.[18]

Harden and **Zilva** and also **Drummond**[19] have concluded that this independence of the rat is apparent rather than real, since rats supplied with orange and lemon juice in addition to a diet containing *A* and *B* were distinctly better nourished than those to which this addition had not been given. With reference to this consideration, **Osborne** and **Mendel**[20] suggest that the fruit product fed as a supply of *C* may have functioned merely as an additional source of *B*, which is present in such products, and thus promote the rate of growth by the increment of a factor other than the antiscorbutic. Improved growth in rats has been observed when the supply of vitamin from yeast was increased. Without a demonstration that more yeast, which is not regarded as an antiscorbutic, or some other source of *B* should fail to accomplish what is claimed for orange juice added to yeast, the conclusion of **Harden, Zilva** and **Drummond** is not convincing. The recent work of **Parsons**[21] casts new light upon the question. The curative effects of feeding rat-livers to guinea pigs, which had developed scurvy, were investigated and this material was found rich in *C*. Practically no difference could be detected in the antiscorbutic content of the livers of rats which had been kept on a diet devoid of *C* and those which had been given orange juice to supply this factor. **Parsons** states:

> It seems entirely improbable that the presence of this factor in the bodies of these animals should be accidental, especially since the amounts in the bodies of the two sets of rats approximate each other so closely. A synthesis of this factor is indicated, both by these results, and by the excellent growth and reproduction obtained in rats by the feeding of rations . . . in which no constituent has been shown to allay scurvy symptoms in the guinea pig. Closely allied to this hypothesis of a synthesis is the possibility that the rat can utilize some slightly different inactive forms of the antiscorbutic substance

[18] **McCollum** and **Parsons,** J. Biol. Chem. 44, 603, 1920.

[19] **Harden and Zilva,** Bioch. J. 12, 408, 1918; **Drummond,** *Ibid.* 13, 77, 1919.

[20] **Osborne and Mendel,** J. Biol. Chem. 41, 554.

[21] **Parsons,** J. Biol. Chem. 44, 587, 1920.

into which the active form perhaps might pass on aging, drying, or through some other condition, and from which it might be rapidly changed in such physiological processes as the sprouting of seeds. An alternative explanation is that the rat has a very definite food requirement for the antiscorbutic factor but that this is quantitatively very small in comparison with the requirement of the guinea pig and has been met in this experiment by the content of the soy bean rations used. . . . Some constituent of this diet might presumably carry a sufficient quantity of the antiscorbutic factor for the requirement of the rat, but escape detection in the light of the much larger requirement of the guinea pig. But if this explanation is accepted it is surprising to find such an abundance of the factor in the bodies of the rats fed on the scorbutic soy bean diet. It would be logical to assume that an extremely small requirement for a food factor would not necessitate the presence of a relatively large supply of that factor in the body of the animal. It is conceivable that the rat has acquired not only a lowered requirement but also a phenomenal capacity to store the antiscorbutic substance through a biological adaptation to a food supply which over long periods of time is very deficient in this substance.

In this connection note should be made of **Funk's** experiment,[22] in which the extract of tissues of birds which had been killed in the last stages of polyneuritis were found to be as efficacious for the cure of this disease as tissue-extracts from healthy birds.

Preliminary experiments in an investigation aimed to test the comparative amount of antiscorbutic substance in eggs and in newly-hatched chickens appear to give some additional weight to the probability of a synthesis of antiscorbutic substance by certain animal tissues, or a utilization of forms other than those available to the guinea pig.

The specific diseases known as the "deficiency diseases" which result from prolonged restriction in or complete deprivation from vitamins are discussed in detail elsewhere. Here it is sufficient to summarize the symptoms which have been observed in animals and human beings as the result of limitation in the vitamin content of the diet.

The most obvious symptoms of deficiency of *A* shown by young animals are lack of growth, diminished appetite, decline and wasting of the tissues to emaciation, sometimes accompanied by diarrhoea, weakness, lack of resistance to infection and disease, and ultimate death.[23] An inflamed condition of the eyes is very commonly reported (for discussion see p. 234 and ff.[24]).

[22] **Funk,** Zeit. f. Physiol. Chem. 89, 373, 1914.

[23] **Osborne** and **Mendel,** J. Biol. Chem. 16, 431; 17, 401, 1914.

[24] **Drummond,** Lancet, 1918, ii, 482; Bioch. J. 13, 95, 1919. **Osborne** and **Mendel,** 20, 379, 1915. See also **Steenbock,** Sci. Monthly, 7, 179, 1918.

Mellanby,[25] while not denying the necessity of *A* for growth, believed that the amount of growth of a puppy has no relation to the amount of *A* in the diet, although a small minimal amount may be necessary. He notes the possibility that puppies have a sufficient store of *A* in their own tissues to allow of growth for several months, independently of what may be in the diet. "Large and rapidly growing puppies require more of the antirachitic factor to prevent rickets If, therefore, this factor is identical with *A*, the presumption is that the function of *A* in the diet of puppies is not so much to ensure growth as to promote correct growth; and the greater the amount of growth in any period, the greater is the amount of fat-soluble *A* necessary to keep it along normal lines. If this view is correct it can hardly be claimed that *A* is in any different category from the point of view of growth than is the antiscorbutic factor, for even in the absence of this latter the rate of growth diminishes and there is often rapid loss of weight."[25a]

Davis[26] has carried on a series of long-time experiments in which several generations of rats were kept on a diet deficient in *A*. The ration used consisted of crushed oats, polished rice and skimmed milk *ad libitum* and a limited amount of cooked potato and egg white. On this ration the half-grown males grew at 80 to 90 per cent of the normal rate for three months. After a period of maintenance they became emaciated and died. None lived longer than eight months.

Most of the females reared one litter and a few reared two on the ration. All but three of the litters were born in the first two months. A second litter of four young was born when the mother had been 148 days on the ration. A female from this lot reared a litter of two young. She was 135 days old at parturition, which made 283 days since the beginning of the experiment. When the young were 25 days old they were discontinued. This made 308 consecutive days for the three generations. Distended abdomens were conspicuous in all of the young born on the ration. In some cases this disappeared after four to five months. Xerophthalmia which **Osborne** and **Mendel** and also **McCollum** and **Simmonds** have reported in the absence of the fat-soluble vitamin appeared at four to five months in the rats born on the ration. In less than 200 days all of the animals had either died or were chloroformed in an emaciated condition. In the litter of 11 young of the second generation born on the ration, in addition to the distended abdomen, and xerophthalmia, small haemorrhagic spots appeared on the tails, and when they were about 30 days old the end of the tails dropped off. The eyes were pale and a blood count showed 4,000,000 to 5,000,000 red blood cells in contrast to a normal count of 8,000,000. A post-mortem examination of the rats put on the ration as well as those born on the ration showed the stomach and intestines filled with gas, the intestines

[25] **Mellanby**, Lancet, 1919, i. 407.

[25a] Attention is called by **Potter** (Intern. J. Pub. Health **1**, 86, 1920; Expt. Sta. Record **44**, 62) to the apparent diversity of opinions and experimental facts in recent contributions to the literature of vitamins and to the possibility of harmonizing some of these diversities.

[26] Private communication, 1920. See also **Davis and Outhouse**, Am. J. Dis. Child. **21**, 307, 1921.

dull and brown, and no introperitoneal fat visible. Autopsy of the second generation born on the ration revealed very marked anaemia. The lungs were white and the liver and kidneys were sand color. The bones were very fragile. There was no apparent beading of the ribs.

A histological examination was made on rats from most of the litters. The organs examined were heart, spleen, liver, lung, pancreas, kidney, and testes. The tissues were removed immediately at autopsy and fixed in Zenker's fluid, imbedded in paraffin, cleared in xylol, and stained with haemotoxylin and eosin, a stain which gives only the marked differences. The examination showed that there were very few changes in the tissues on this ration. The heart, pancreas, liver and testes remained normal in most cases. Slight changes were found in the kidney and spleen. Congestion and chronic inflammation was frequently found, indicating, in all probability, a secondary infection, although an effort was made to discard without microscopical examination all those which gave evidence of infections of the respiratory tract. The spleen showed very slight changes. There was congestion with frequent dilation of venous sinuses. In some the congestion was so marked as to mask the structure. Necrosis or degeneration of splenic reticulum was not observed. There seemed to be no relationship between the degree of congestion and the duration of the experiment. The main finding in the kidney was cloudy swelling of the parenchyma of the collecting tubules. This was more marked in the tissues of the second generation born on the ration. An albuminous precipitate was present in the tubules of some. Congestion also was found. In places the cells of the tubules appeared skeletonized, and the glomerular tufts slightly shrunken. The liver appeared normal in all cases. Necrotic and degenerative changes were absent. In one lot there was a total absence of red blood cells. Otherwise the structure was normal.

Drummond, Emmett and **Allen**[27] had previously reported that they could find no outstanding pathological effects from deficiency of *A*.

It has been suggested that the so-called "war oedema" may be due to lack of vitamins; and there is evidence which seems to indicate that the vitamin *A* may be the factor involved,[28] although **Madsen**[29] was inclined to regard this disturbance as a rudimentary form of beriberi.

Hess, McCann and **Pappenheimer**[29a] fed young rats a diet complete except for a lack of the fat-soluble vitamin. They invariably failed to grow and generally developed keratitis. The keratitis developed less frequently when the ration included orange juice. If this diet is continued for a period of months the animals die, either

[27] **Drummond,** Bioch. J. 12, 95; **Emmett** and **Allen,** Proc. Soc. Biol. Chem. J. Biol. Chem. 41, iii, 1920.

[28] **McCarrison,** Ind. J. Med. Res. 6, 275, 550, 1919; **Bigland,** Lancet, 1920, 1, 243.

[29] **Madsen,** Ugesk. f. Laeger, 80, 613, 1918.

[29a] **Hess, McCann** and **Pappenheimer,** J. Biol. Chem. 47, 395, 1921.

of inanition or, more often, of some intercurrent infection. The skeletons of such rats show no gross changes whatsoever. Microscopic examination of the bones of twenty-two rats on a ration of this character presented definite signs of a lack of active osteogenesis, but in no instance lesions resembling rickets. In view of these results and their conformity with previous experience in regard to infantile rickets, Hess, McCann and Pappenheimer are of the opinion that this vitamin cannot be regarded as the antirachitic vitamin, and that, if the diet is otherwise adequate, its deficiency does not bring about rickets.

Harden and **Zilva** [30] fed monkeys on a diet free from *A* and low in fat, but complete in all other respects. Of three monkeys one died, showing signs of malnutrition and very emaciated; while a second lost weight and displayed symptoms of oedema 289 days after the experiment had been commenced. **McCarrison** has observed that oedema is invariably associated with massive enlargement of the adrenals in pigeons fed on autoclaved rice, and that fresh butter contains some substance which tends to protect against oedema, and which was not present in a coconut oil. This hypothetical substance appears to exert its protective action by maintaining the adrenaline content of the adrenals at a low level. Butter is richer in this substance when cows are fed on green fodder than when fed on dry fodder.

Kohman [31] discounts the influence of vitamins in the etiology of oedema and asserts that a combination of low calories, low protein, and excessive fluid intake is sufficient of itself to produce symptoms corresponding in all respects to those of war oedema.[32]

The relation between lack of vitamin *B* and the nervous disorder known as beriberi in man and polyneuritis in the lower animals is well recognized, but it is very probable that this acute stage is much less serious on the whole than the less obvious disorders which may find their origin in the same cause. **McCarrison**,[33] who has made a very extensive study of the results of deficiency in this factor, asserts that complete absence of vitamin *B* from food is of less practical importance from the point of view of the production of disease in human beings than its subminimal supply. The former leads to rapid dissolution and death, the latter to slow dissolution and disease,

[30] **Harden** and **Zilva**, Lancet, 1919, ii, 780.

[31] **Kohman**, Am. J. Physiol. 51, 378, 1920.

[32] See also **Bigland**, Lancet, 1920, i. 243; **Menzies**, 26, 350, and **Mayer**, J. Am. Med. Assoc. 74, 931, 1920.

[33] **McCarrison**, Ind. J. Med. Res. 6, 375, 550, 1919; 7, 167–325, 633, 1920; Brit. Med. J. 1919, 1, 177; II, 36; 1920, I, 249; 822; II, 236; Proc. Roy. Soc. 91B, 103, 1919–20.

suggesting perhaps some explanation of the great mass of ill-defined gastro-intestinal disorders. From his observations, involving detailed post-mortem examination on some hundreds of pigeons, and from his clinical experience, **McCarrison** has been led to draw the following conclusions:

"The absence of certain accessory food factors, improperly termed 'antineuritic,' from the dietary, leads not only to functional and degenerative changes in the central nervous system, but to similar changes in every organ and tissue of the body. The morbid state to which their absence gives rise is not to be interpreted as only neuritis. The observed effects are due to chronic inanition, derangement of the function of the organs of digestion and assimilation, functional disturbance of the glands of internal secretion, malnutrition of the nervous system, and hyperadrenalinemia. Certain organs undergo hypertrophy, other atrophy."[33a]

Those which atrophy, and in order of severity named, are thymus,[34] testicles,[35] spleen, ovary,[36] pancreas, heart,[37] liver, kidneys, stomach, thyroid[38] and brain. The pituitary gland showed in adult birds a slight tendency to enlargement in males only.

Funk and **Douglas** report that a large number of the secretory cells of the pituitary gland are destroyed. The enlargement of the adrenals is a true hypertrophy in so far as it is associated with proportional increase of the adrenaline content of the gland.[39] The quantity and quality of adrenaline in the hypertrophied organ is, area for area, approximately the same as that found in the adrenals in health. The hypertrophy is equally well marked in both sexes. **McCarrison** found that oedema was invariably accompanied by great hypertrophy of the adrenal glands, while 85 per cent of all cases having great hypertrophy of these organs had oedema in some form. The amount of adrenaline, as determined by physiological methods, in such cases has been considerably in excess of that found in cases not presenting this symptom, and greatly

[33a] **Osborne** and **Mendel** (Proc. Soc. Exptl. Biol. Med. 17, 46, 1919) conducted experiments on rats in whose diet fruits and fruit juices furnished the sole source of vitamin *B*. More than 5 g. per day of fresh apples and pears avert the characteristic decline in weight observed where vitamin-free diets devoid of this vitamin are used. Fruits are too bulky to permit the feeding of more than 10 g. per day. Ten c. c. of orange juice per day suffice to promote considerable growth. The inner peel of the orange apparently contains both kinds of water-soluble vitamin.

[34] See also **Emmett** and **Allen,** Proc. Soc. Biol. Chem. J. Biol. Chem. 41, liii, 1920; **Funk** and **Douglas,** J. Physiol. 47, 475, 1914.

[35] See also **Dutcher,** Proc. Nat. Acad. Sci. 6, 10, 1920. See also **Funk** and **Douglas,** l. c.

[36] See also **Funk** and **Douglas,** l. c.

[37] See also **Dutcher,** Proc. Nat. Acad. Sci. 6, 10, 1920.

[38] See also **Funk** and **Douglas,** l. c.

[39] See also **Emmett** and **Allen,** l. c.

in excess of that found in normal adrenals. Inanition gives rise to a similar state of adrenal hypertrophy and to a similar state of atrophy of the other organs, the brain itself possibly excepted. The oedema of inanition and of beriberi is believed to be initiated by increased production of adrenalin, acting in association with malnutrition of the tissues. Failure of circulation and venous stasis may subsequently contribute to it, and age is an important factor determining its occurrence. This finding is held to account in a great measure for the occurrence of war oedema among prisoners of war in Germany.

Gastric, intestinal, pancreatic, and biliary insufficiency are, according to **McCarrison,** important consequences of a diet too rich in starch and too poor in vitamins and other essential constituents of the diet. A state of acidosis results from the absence of so-called antineuritic vitamin, which state is due to imperfect metabolism of carbohydrates and to acid fermentation of starches in the intestinal tract. Chemically it is evidenced by progressive slowing and deepening of the respirations. He suggests that some of the obscure metabolic disorders of childhood be examined from this point of view as well as from that of starvation of the glands of internal secretion.

Great atrophy of muscular tissue results from a deficiency of accessory food factors. **McCarrison** holds that this is due, in part, to disturbance of carbohydrate metabolism in consequence of disordered endocrine function, and in part to the action of the adrenals in supplying blood to the vegetative organs of the body at the expense of the muscles. The central nervous system atrophies little; paralytic symptoms when they occur are due mainly to impaired functional activity of the nerve cells, much more rarely to their degeneration.

Pigeons on a diet of autoclaved milled rice (deficient in both *A* and *B*) showed congestive and atrophic changes in all coats of the bowel, lesions of its neuro-muscular mechanism, impairment of digestive assimilative processes and failure of its protective resources against infection. Great susceptibility to various septicaemic and tuberculosis infections were also shown. **McCarrison**[40] suggests that certain gastro-intestinal disorders in the human subject (e. g. mucous disease, celiac disease and chronic intestinal stasis) may owe their origin to subminimal supply of accessory food factors protracted over long periods of time.

Since both inanition and a diet too rich in starch and too poor in vitamin lead to depression of biliary, pancreatic, and gastro-intestinal functions, it follows that if these functions are exposed in addition to toxic or bacterial infection their depression will be manifestly greater. The many toxic influences to which human beings are subject under conditions of food deficiency must play an important part in further depressing these organs and tissues on which normal

[40] **McCarrison,** Brit. Med. J. 1919, II, 36.

metabolism are dependent. That abnormal putrefaction in the intestine actually exists in beriberi is shown by the increased indican content of the urine. The toxic products of the intestinal bacteria or parasites may thus assume a rôle of high importance in the genesis of morbid states which were no doubt initiated by the dietetic defect. Vitamin deficiency renders the body very liable to be overrun by a rank growth of bacteria.[41]

Monkeys fed on a diet of autoclaved milled rice, with or without the addition of butter-fat, died within two or three weeks, death being more rapid in the group given butter-fat than among those fed on rice alone. There was no evidence of polyneuritis.[42] the symptoms noted being progressive anaemia, gastro-intestinal disorders, and progressive asthenia. The stomach and abdominal walls were greatly thinned and the whole gastro-intestinal tract showed congestive, necrotic, and inflammatory changes, with degenerative changes in the neuro-muscular mechanism, leading to dilation of the stomach and ballooning of areas of the intestine.[43]

This also points to the conclusion that dietaries which are deficient in vitamins and protein, and at the same time excessively rich in starch or fat, or both, are potent sources of disease, and especially of gastro-intestinal disease. An excess of fat, in association with a deficiency of *B* vitamin and protein and superabundance of starch, is peculiarly harmful.[44] Under such conditions the invasion of the blood and tissues by bacteria is greatly favored.

Observations very similar to those of **McCarrison** were made by **Emmett** and **Allen** [45] on rats which had been deprived of *B*. The various tissues examined histologically were: Thymus, thyroid, pancreas, testes, ovaries, adrenals, liver, spleen, ilium, colon, kidneys, heart, lungs, brain, optics, lower cord, and sciatic nerves. The most noticeable changes were found in the almost complete atrophy of the thymus; hypertrophy of the adrenals; passive congestion; fatty infiltration; and at times fatty degeneration in the liver [46] and some atrophy of muscle fibers of the heart. In addition there was more or less passive congestion in the pancreas, spleen, ilium, colon, kidneys, and lungs.

Vezar and **Bogel**,[47] in studying the effect of various accessory food materials, found that the alcoholic extract of vitamin *B* from bran gave a marked vasoconstriction, which was, however, not obtained with an aqueous extract of the same material, and which was there-

[41] See also, **Drummond**, Bioch. J. 13, 55, 1919.
[42] But compare **Gibson**, see p. 207.
[43] See also **Hewlett** and **de Korte**, Brit. Med. J. 1907, 11, 201.
[44] See also **Emmett** and **Allen**, J. Biol. Chem. 38, 325, 1919.
[45] **Emmett** and **Allen**, J. Biol. Chem. 41, liii, 1920.
[46] See also **Funk** and **Douglas**, J. Physiol. 47, 475, 1913-14.
[47] **Vezar** and **Bogel**, Bioch. Zeits. 108, 1920.

fore probably due to something other than vitamin *B*. Similarly, extract of *A* from butter gave a vasodilation, but the same effect was obtained from aqueous extract of butter, which renders it improbable that vitamin *A* was the potent factor.

Atrophy of the sexual glands as a result of vitamin deficiency has been noted repeatedly,[48] and would account for the sterility frequently observed among animals on a deficient diet.[49] **McCarrison**[50] says: "Profound atrophy of the reproductive organs is an important consequence of vitamin deficiency. It leads to cessation of the function of spermatogenesis. In the human subject such degrees of atrophy would result in sterility in the males and in amenorrhea and sterility in the females. This finding is held to account in great measure for the occurrence of "war amenorrhea." In this connection it may be noted that **Miles**[51] has reported a case where 24 young men kept for four months on a restricted diet reported without exception a pronounced reduction of sexual instinct.

Dutcher and **Wilkins**[51a] have observed that the testes of cockerels did not develop on a diet of polished rice, but when the rice was supplemented with small amounts of green alfalfa there was no atrophy of the testes and when alfalfa was added to the diet of cockerels which had been on a rice diet for 36 days the atrophied testes began to increase in weight. That the observed atrophy is not merely the result of general inanition was indicated by its appearance while the birds were actually increasing in body weight and by the small amounts of alfalfa necessary to produce improvement.

Madsen[52] suggests that a lack of vitamins is a factor in chlorosis, anemia, neurasthenia, and vasomotor disturbances. This is borne out by the statement of **McCarrison** (l. c.) that the red cells of the blood are diminished by about 20 per cent after a period of vitamin deficiency.

Mattill[52a] states that the total solids, urea and non-protein nitrogen of the blood are normal in rats deprived of *B*, while the creatinine is at the fasting level, and the creatine slightly higher than in fasting animals.

[48] **Funk** and **Douglas**, J. Physiol. 47, 475, 1913–14; **McCarrison**, l. c.; **Wilkins** and **Dutcher**, Sci. 52, 393, 1920.

[49] **Drummond**, Bioch. J. 12, 25, 1918; **Abderhalden**, Arch. ges. Physiol. 175, 187, 1919.

[50] **McCarrison**, Brit. Med. J. Feb. 15, 1917.

[51] **Miles**, J. Nerv. and Ment. Dis. 49, 208, 1919. See also **Reynolds** and **Macomber**, J. Am. Med. Assn. 77, 169, 1921, on defective diet as a cause of sterility.

[51a] **Dutcher** and **Wilkins**, Am. J. Physiol. 57, 437, 1921.

[52] **Madsen**, Ugesk. f. Laeger, 80, 613, 1918.

[52a] **Mattill**, Sci. Aug. 26, 1921, 176.

In addition to its antineuritic properties vitamin *B* exerts a pronounced effect on growth.[53] Parallel with increased growth is markedly increased appetite.

Drummond[54] noted that the food consumption of rats fed upon the deficient diet is low, being in all probability reduced to that sufficient to supply the calorific requirements of maintenance, and adds that an increased food consumption may be brought about by addition of flavoring agents, such as meat extracts, to the diet, but unless this agent contains the water-soluble substance it causes no increase of growth. "Addition to an inadequate diet of extract containing *B* causes greatly increased food intake, immediately followed by growth. The amount of growth is, within certain limits, proportional to the amount of accessory substance added, provided the diet is adequate in other respects. There is evidence that the length of time a rat is able to maintain himself upon a diet deficient in water-soluble substance without suffering serious loss of body weight is directly proportional to the age at which the restriction is imposed."

Osborne and **Mendel**[55] remark in this connection:

> If for any reason the food intake falls below the normal, the amount of vitamin consumed likewise falls, with the effect of impairing the condition and further reducing the food intake. Thus the influence of the vitamin is continuously diminishing and consequently also the food intake. Whether the water-soluble vitamin simply increases appetite and hence promotes growth, or whether it supplies some essential chemical factor which renders growth possible cannot be determined by any data heretofore recorded. The fact remains, however, that the addition of vitamin to a ration containing too little of this factor is followed by a very marked increase in food intake with accompanying rapid gain in weight. On the other hand, loss of weight on vitamin-poor diets almost invariably precedes a lessened intake of food. This suggests that the impaired health of the animal is due to insufficiency of an essential food factor rather than to an insufficient quantity of food. Conversely, when the water-soluble vitamin is increased in quantity, after a decline on a vitamin-poor diet, the increased food intake which follows is perhaps due to an improved physical condition caused by the vitamin, for the resulting rapid recovery in body weight must necessarily be accompanied by a correspondingly greater food intake.

Hopkins[56] is of the opinion that the desire for food is a consequence of the increased food requirements due to the laying on of

[53] For discussion as to whether there are one or two vitamins involved here see Chapter VIII.

[54] **Drummond**, Bioch. J. 12, 25, 1918.

[55] **Osborne** and **Mendel**, J. Biol. Chem. 37, 194, 1919.

[56] **Hopkins**, J. Physiol. 44, 442.

new tissue. Little as we know in detail with regard to the factors (other than aesthetic ones) which control appetite, it is physiologically axiomatic that the rate of the metabolism and, in immature animals, the rate of growth, are of fundamental importance in determining the amount of food that a healthy animal will in the long run voluntarily consume. What in this sense is a diminution of appetite determines the diminished relative consumption of food observed when a small animal grows larger; the proportionate rate of metabolism and the relative velocity of growth are both diminished in the larger animal, and the consequently lessened demands are, under natural conditions, followed by a smaller relative consumption. Again, while the young animal eats sufficient for growth as well as for maintenance, the adult is content with an intake sufficient for maintenance alone. It may be argued that the voluntary intake is directly affected by the presence or absence of the growth impulse in the tissues; but it appears more likely to be the actual occurrence of the growth processes, the laying on of new tissues with its own metabolic demands, that affects the instinctive appetite. Only those perhaps who have had the experience of feeding animals with excess of food, and have noted the amount eaten for considerable periods, will realize how well adjusted, under normal circumstances, is the instinctive appetite to the physiological needs.[57]

If then a factor or factors essential to growth be missing from, or deficient in, a dietary, the consequent arrest of, or diminution in, growth energy may diminish the instinctive consumption of food, while the supply of such factors may increase consumption as an indirect result of a direct affect upon growth.

Karr [58] found that feeding dogs on a diet very deficient in vitamins resulted in decreasing appetite to the point of ultimate refusal to eat at all, unless vitamin was added. He states that a rather definite minimum amount of vitamin *B* seems to be necessary to sustain the desire to partake of food which is otherwise adequate for the nutrition of the animal. The actual amount of vitamin required varied with different animals, 0.5 g. of brewery yeast for instance being sufficient for some, while others required three times as much. That lack of vitamin is the direct cause of the failing appetite was shown by the

[57] See also **Osborne** and **Mendel**, J. Biol. Chem. 20, 359, 1915.

Comparative calorimetric studies of fasting and avitaminosis have been carried out by **Novaro** (Pathologica 12, 133, 1920; Physiol. Abstracts 5, 555, 1921).

[58] **Karr**, J. Biol. Chem. 44, 255, 1920.

fact that there was no such failure when the requisite amount of vitamin was given from the start as an addendum to the diet.

Cowgill[58a] has confirmed the observations of **Karr**, and demonstrated that alcoholic extracts of wheat embryo, rice polishings and navy bean contain the appetite-promoting factor.

In addition to the well-known symptoms of scurvy resulting from deficiency of *C*, **McCarrison**[59] has noted the following results determined from the histological examination of guinea pigs which had been kept on a diet of crushed oats and autoclaved milk: The size and weight of the adrenal glands increased, accompanied by degenerative changes in the cellular elements of the adrenal cortex and medulla and marked diminution of the adrenaline content. The bladder showed congestion of the mucous and muscular coats and degenerative changes of the epithelium, with hemorrhagic infiltration. In many cases there was considerable enlargement of the thyroid, the result in the main of congestion and hemorrhagic infiltration of the tissues.

A connection between calcium metabolism and the vitamin content of the diet is indicated by the work of several investigators. **Mellanby**[60] concluded from his study of puppies that substances containing and associated with *A* are particularly concerned in the calcification of the bones and teeth, a point of view which is confirmed by the work of **Shipley, Park, McCollum, Simmonds** and **Parsons**,[61] who found the cartilage and adjacent portions of the metaphsis of the long bones of the extremities of rats could be rendered entirely free from calcium by feeding diets low in *A*. After about 40 days on this ration, when the symptoms characteristic of lack of *A* were well developed, two per cent of cod liver oil was incorporated in the diet, with the usual beneficial effects. After a few days the animals were killed and their bones compared with those of control animals which had received no oil. While no gross differences could be detected, microscopic examination showed that those to whose diet the oil had been added showed a line of freshly laid down lime salt in the matrix of the proliferative zones, while the epiphyseal cartilages of the controls were calcium-free. **Telfer**[61a] on the other hand states that an experiment with an 8-months-old infant on the in-

[58a] **Cowgill**, Am. J. Physiol. 57, 420, 1921.

[59] **McCarrison**, Ind. J. Med. Res. 7, 188, 284, 633, 1920.

[60] **Mellanby**, Proc. Soc. Physiol. J. Physiol. 52, liii, 1919.

[61] **Shipley, Park, McCollum, Simmonds,** and **Parsons**, J. Biol. Chem. 45, 343, 1920.

[61a] **Telfer**, Proc. Physiol. Soc., J. Physiol. 54, cv, 1920.

fluence of cod liver oil and butter-fat on the retention of calcium and phosphorus gave no support to the theory that these substances in the diet specifically affect calcium retention.

It is believed by some observers that other vitamins besides *A* may be concerned in the deposition of calcium. **McCarrison** notes that lack of *B* causes thinning of the bones and decrease in the bone marrow. Rarefication and fragility of the long bones and enlargement of the junctions of the ribs and cartilages are commonly reported as symptoms of scurvy resulting from deficiency of *C*.

The recent work of **Mellanby** [62] and **Howe** [63] on the connection between tooth formation and the vitamin content of the diet is of great interest. According to **Mellanby,** a diet, containing an abundance of food with which *A* is associated, allows the development of sound teeth in puppies.

> A diet otherwise adequate but deficient in such substances brings about the following defects in puppies' teeth: delayed loss of deciduous teeth, delayed eruption of permanent dentition, irregularity in position and over-lapping, especially of incisors, partial absence of or very defective enamel, and low calcium content. The deficiency in calcium salts may even result in teeth being so soft that they can be cut with the scalpel. The evidence makes it clear that this is an instance of diet affecting the teeth from inside and is independent of bacterial sepsis and other conditions associated with food. These results cannot be considered as due to acute illness of malnutrition, for the improvement of the teeth on adding substances containing *A* is as characteristic as the deleterious effect of the deficient diet, and there is evidence that the defective teeth are most pronounced in rapidly growing puppies.

Mellanby points out that the crowns of the deciduous teeth of children are for the most part developed before and during the first few months after birth. In order to supply the offspring, the mother must have an adequate supply of *A* in the diet.

Howe found that deficiency in *C* may also cause serious defects in the teeth. Guinea pigs which have been kept on a scorbutic diet for a considerable period of time show effects which simulate pyorrhea more closely than does any other condition that has been produced in animals by experimental conditions. The teeth become elongated, irregular, and very loose; the gums become red and spongy, and extensive absorption of the alveolus occurs; the dental pulps show changes before the grosser indications appear. Other effects produced are loss of luster by the hair, and sensitive, slightly swollen,

[62] **Mellanby,** Lancet, 1918, ii, 765.
[63] **Howe,** Dental Cosmos, 62, 536, 1920; J. Home Ec.

rheumatic joints. He believes that a scorbutic diet may produce the same effects in man.

Various attempts have been made to relate the vitamins *A* and *B* with phosphorus metabolism, but direct and satisfactory experimental evidence is lacking.[64]

The close association of *A* with fats in nature suggests that this vitamin might function in fat metabolism, but no direct connection could be traced by **Drummond**,[65] who found that rats on a diet lacking *A* are capable of absorbing large amounts of free fatty acids and of effecting synthesis of these into neutral fats over a considerable period of time. He concludes, therefore, that this factor plays no important part in the synthesis of fatty acids into fats following absorption. The power of absorbing fatty acids and effecting fat synthesis is apparently retained after the animal has begun to show the characteristic symptoms of lack of *A*. **Drummond**[66] suggests that fat is dispensable in the diet and only useful for supplying *A*, a hypothesis which finds support in the experiments of **Osborne** and **Mendel**[67] in which rats on a diet containing mere traces of fat if any, but supplied with an adequate amount of *A*, grew normally and appeared as well nourished as the control animals which received liberal quantities of *A*. These experiments would seem to indicate that fats in themselves are dispensable constituents of the diet, except in so far as they serve as carriers of *A*.

That vitamin *B* is concerned, directly or indirectly, in fat metabolism is indicated by the experiments of **McCarrison** with monkeys, already mentioned, and by those of **Funk** and **Dubin**,[68] who showed that the growth of rats on a high fat diet was inhibited more completely by lack of *B* than was the case with those on a carbohydrate diet, and these in turn grew more slowly than a group on a protein (meat) diet. Of the two carbohydrates, sugar and starch, the latter appeared to require more vitamin for its metabolism than did the former. With pigeons on the contrary a high fat diet delayed the appearance of polyneuritis while a high percentage of carbohydrate had the greatest effect in hastening its onset. This effect of carbohydrate in increasing the antineuritic requirement has been repeatedly

[64] **Funk**, J. Physiol. 44, 50, 1911; **Simpson and Edie**, Ann. Trop. Med. Par. 5, 313, 1911-12; **Osborne and Mendel**, J. Am. Med. Ass. 69, 32, 1917; **Padua**, Phil. J. Sci. 14, 481, 1919; **Jelliffe**, J. Nerv. Ment. Dis. 49, 522, 1919.

[65] **Drummond**, Bioch. J. 13, 95, 1919.

[66] **Drummond**, Bioch. J. 13, 95, 1919.

[67] **Osborne and Mendel**, J. Biol. Chem. 45, 145, 1920.

[68] **Funk and Dubin**, Sci. 52, 447, 1920.

observed by **Funk**[69] and was confirmed by **Braddon and Cooper,**[70] but is denied by **Eikjman** and **Vedder.**

Vedder[71] asserts that the rapidity with which polyneuritis develops bears no relation to the amount of rice eaten but depends on the idiosyncrasy of the fowl to this deficiency. "Experiments performed to determine the relation of the antineuritic vitamin to carbohydrate metabolism by hand-feeding birds with varying amounts of carbohydrate foods are fallacious if the amounts fed are too excessive or too minute. The birds receiving too much die from overfeeding and the birds receiving too little die of starvation. Fowls fed on sterilized meat or sterilized egg will develop neuritis. When fowls are fed on equal parts of sterilized meat and rice or sterilized egg and rice, they develop neuritis more slowly than when fed on sterilized egg or meat alone. These experiments indicate that the antineuritic vitamin is not concerned in carbohydrate metabolism."

Cramer[72] has observed that when the vitamins are withdrawn from the diet the peculiar glandular adipose tissue (which appears to be functionally related to the thyroid and adrenal glands) disappears, although if vitamins are supplied this is retained even under the conditions which bring about the disappearance of the ordinary adipose tissue.

Funk[73] has stated that all animal organisms on a diet deficient in vitamins will in time come into a negative nitrogen balance, even though the diet is entirely adequate in regard to its amino acid content, and **Degrez** and **Bierry**[74] assert that in order to maintain nitrogen equilibrium in absence of vitamins it is necessary to reduce the protein and increase the sugar in the diet, the minimum protein, fats, and carbohydrates permissible in a diet deficient in vitamin being controlled by the chemical nature of the particular class of nutrient and the ration of the other two classes present. **Drummond,**[75] on the other hand, reports that the only deviation from the normal nitrogen metabolism shown by rats on a diet deficient in *B* was the appearance of creatinuria which accompanied the wasting of the skeletal muscles. **Karr**[76] has recently found that the nitrogen utilization in the digestive tract is unaffected by the absence of *B* and that the intermediar metabolism of nitrogen is apparently not materially affected by this lack.

[69] **Funk,** Proc. Physiol. Soc. J. Physiol. 47, xxv, 1913; *Ibid.,* Z. f. physiol. Chem. 89, 378, 1914; **Funk and Schonborn,** J. Physiol. 48, 328, 1914; **Funk,** *Ibid.,* 53, 247, 1919.

[70] **Braddon and Cooper,** J. Hyg. 14, 351, 1914.

[71] **Vedder,** J. Hyg. 17, 1-9, 1918.

[72] **Cramer,** Brit. J. Exp. Path. 50, 184, 1920.

[73] **Funk,** Die Vitamine, Wiesbaden, 1913; J. Biol. Chem. 27, 173. 1916.

[74] **Degrez and Bierry,** C. r. 170, 1209, 1920.

[75] **Drummond,** Bioch. J. 12, 25, 1918.

[76] **Karr,** J. Biol. Chem. 44, 277, 1920.

In a study of the growth of tumors in mice **Van Alstyne** and **Beebe**[77] found that 24 out of 26 animals on a diet of casein, lard and lactose grew a tumor with fatal results, while on a diet of casein and lard only two were killed by the tumor. **Sweet, Corson-White** and **Saxon**[78] argued that this result was due to the growth-stimulating effect of the accessory substance introduced with the lactose.[78a] From their investigations they concluded that tumor cells are subject to the same laws of growth as normal somatic cells, the only difference being that the tumor cell possesses stronger avidity for the unknown substance in the diet which is essential for cell growth, and will therefore persist in developing even though the individual is losing weight.[79]

While data is constantly accumulating regarding the indispensability of the vitamins and the disastrous effects due to scarcity of these factors, hypotheses as to their precise physiological function are few and vague. **McCarrison**[80] believes that the whole morbid process indicative of vitamin deficiency is the result of nuclear starvation of all tissue cells, since even the adrenals, which alone of all the organs of the body undergo enlargement, show on sectioning, changes in some of their cells indicative of nuclear starvation. This, however, merely changes the form of the question and leaves us asking what derangement of functions and processes causes this nuclear starvation. **Portier's** theory[80a] that the vitamins impregnate nuclear substance bringing about "nuclear fecundation" and thus furnishing the cells with an impetus without which they cannot live, is equally vague.

Arguments based on the distribution of the vitamins in the tissues are unconvincing, partly because of the contradictory data given by different observers, and partly because of the variety of interpretations possible for the accepted results.

McCollum has attempted to correlate high vitamin content with actively functioning cells, with the inference that the presence of vitamins in such cells is indispensable for their development.

Steenbock and **Gross**[81] point out that this relation does not by

[77] **Van Alstyne** and **Beebe**, J. Med. Res. 24, 217, 1913–14. See p. 309.

[78] **Sweet, Corson-White** and **Saxon**, J. Biol. Chem. 21, 314, 1915.

[79] See also **Corson-White**, Penn. Med. J. 22, 348, 1919.

[78a] **Hawk, Smith** and **Bergheim** report that strained honey does not appear to contain appreciable amounts of vitamins *A, B* and *C*. Honeycomb contains moderate amounts of *A*. (Am. J. Physiol. 55, 339, 1921).

[80] **McCarrison**, Ind. J. Med. Res. 6, 275, 550, 1919.

[80a] **Portier**, Bull. soc. sci. hyg. 8, 521, 603, 1920.

[81] **Steenbock** and **Gross**, J. Biol. Chem. 40, 503, 1919.

any means invariably hold in the plant kingdom, as is shown by the great variation in the *A* content of similar organs, such as tubers and roots. **Drummond** [82] has shown that actively growing animal tissues such as embryos and tumors do not contain appreciable amounts of *B*. **Swoboda,** using a modification of the **Williams'** yeast test,[83] could detect little vitamin in tissues high in nuclear material such as thymus and lymph gland, while, on the other hand, yeast with its high content of nuclear material is one of the richest sources of vitamin.

Steenbock and **Gross** [84] consider that the vitamin content of liver tissue, instead of being due to or associated with its activity as a glandular organ, might with equal propriety be looked upon from the standpoint of its well known functions as a storage organ, or might possibly be attributed to the absorption of substances not indispensible to the organ but absorbed due to an inefficiency of the mechanism which excludes the entrance of substances in amounts greater than the needs as these substances are brought to it in the portal circulation. Similarly the high vitamin content of the kidney may be said to be due to the temporary retention of the vitamin. **Voegtlin** and **Towles** [85] found from observations made on the vitamin content of the spinal cord that nerve fibres contain the antineuritic substance, mainly in combined form, which is liberated after autolysis of the tissue, and judged from this that the antineuritic substance under normal conditions formed an essential part of the nerve fibre, and that its presence in the nerve tissue in sufficient amounts is essential for proper functioning of this organ. The long list of compounds, unrelated chemically, such as betaine, allantoin, certain pyrimidines,[86] nicotinic acid,[87] adenine,[88] α-hydroxy-pyridine,[89] thyroxin (trihydrotriiodo-oxyindol-propionic acid), pilocarpine, tethelin,[90] α-ketoγ–propylindol, and n-methyl-β-ethylindolinone,[91] which have been found to exert a curative effect upon avian polyneuritis would seem to indicate, however, that the function of the antineuritic vitamin may be merely to provide nerve stimulant, an effect which might equally well be produced indirectly.

Uhlmann [92] concluded that the antineuritic vitamin acts on the

[82] **Drummond,** Bioch. J. 12, 25, 1918.
[83] **Swoboda,** J. Biol. Chem. 44, 539, 1920.
[84] **Steenbock and Gross,** J. Biol. Chem. 40, 503, 1919.
[85] **Voegtlin and Towles,** J. Exp. Pharm. 5, 67, 1913.
[86] **Funk,** J. Physiol. 45, 489, 1912-13.
[87] **Funk,** *Ib.,* 46, 177, 1913.
[88] **Williams and Seidell,** J. Biol. Chem. 26, 431, 1916.
[89] **Williams,** *Ib.,* 29, 504, 1917.
[90] **Dutcher,** J. Biol. Chem. 39, 63, 1919.
[91] **Dutcher, Holm, and Bierman,** Sci. 52, 589, 1920.
[92] **Uhlmann,** Zeitsch. Biol. 68, 457, 1918.

sympathetic nerve endings and thus exerts a controlling influence on the tones of the muscles in the organism. He was led to this conclusion by the observation that vitamin extracts act upon animal tissues very much as do pilocarpine and choline. The intestinal muscles are caused to contract, the action of the heart is depressed, the blood pressure is lowered, and the blood vessels dilated exactly as occurs when the respective tissues are treated with pilocarpine.[93] Moreover, the action of the vitamin, like that of pilocarpine or choline, is antagonised by atropine.

Vedder[94] is of the opinion that the antineuritic vitamin is a building stone which is essential for the metabolism of the nerve tissues, basing his opinion on the following facts:

1. If the supply of this vitamin is cut down by feeding exclusively on polished rice, changes in the structure of the nerve fibres of fowls may be demonstrated after only seven days on such a diet. The evidence of incipient degeneration at such an early date appears to indicate that a certain amount of this vitamin is constantly necessary in order to maintain the nervous system in a healthy condition.
2. The nerve cells from the cord of fowls suffering from polyneuritis gallinarum present changes very similar to those demonstrated in the nerve cells of birds that suffer from fatigue as the result of long flights.
3. Fowls suffering from polyneuritis may be completely cured within a few hours by the administration of the vitamin obtained from rice polishings.

McCollum and **Simmonds**[95] suggest that polyneuritis symptoms in an animal are due to progressive degeneration of the motor cells of the spinal cord. Even at the acute stages, however, there are normal cells which are capable of functioning if sufficiently stimulated, and consequently any substance which supplies the necessary stimulus through its pharmacological action will induce at least temporary relief. **Cramer, Drew** and **Mortram**[95a] have pointed out that in avitaminosis there is extreme reduction of the lymphocytes, both in the lymphoid structures and in the blood, and that this condition is also produced by exposure to X rays and radium. Moreover these agents, if used in sufficient amount produce other effects very similar to those of avitaminosis, loss of weight, degeneration of the seminiferous tubules of the testes, and ultimate death. They suggest that all these agencies may exert a selective action on the lymphoid tissue and that this tissue is more important in the nutrition of animals than has been supposed.

[93] See p. 173, **Vezar** and **Bogel.**

[94] **Vedder,** Proc. 2nd Pan Am. Sci. Cong. Sec. 8, pt. 2, p. 27.

[95] **McCollum** and **Simmonds,** J. Biol. Chem. 33, 55, 1918.

[95a] **Cramer, Drew** and **Mortram,** Lanc. 1921, I, 963.

Lumiere [96] ascribes the death of pigeons fed on polished rice to starvation from lack of appetite, the result of stagnation of the ingested rice from lack of secretions to aid in digesting it and passing it along the alimentary canal. He concludes that the special function of the vitamins is the stimulation of the gastro-intestinal motor functioning and the secretory functions of the glands with an external secretion. Without vitamins the digestive glands cease to function, and the animals succumb to loss of these secretions although their digestive tract may contain abundance of nourishment. On the other hand, animals will succumb if deprived of sufficient nourishment to keep them in health, even if there is abundance of vitamins present. About one-third of the animals under observation developed in addition to the usual symptoms of starvation the paralytic and cerebellar disturbances which have been regarded as characteristic of vitamin deficiency. Prompt recuperation when vitamins are supplied after deficiency disease has developed testifies to their stimulatory power on the digestive glands. The special development of the cerebellum in birds to control orientation in flight requires an extra amount of nourishment, so when nourishment is shut off by lack of vitamins the cerebellum suffers first and the cerebellar symptoms are the first to appear. In support of his view **Lumiere** states that pigeons develop polyneuritis as quickly on a starvation diet but with excess of vitamins as they do on the same diet without vitamins. When rice is supplied to these pigeons in sufficient amounts they recuperate as rapidly as those which have developed polyneuritis through feeding on polished rice do when supplied with vitamins. The disease produced by feeding a ration of 4 g. of polished rice, 2 g. glucose, and 1 g. brewers' yeast per day was cured by increasing the amount of polished rice. On the other hand, **Karr** [97] concludes from the fact that the utilization of nitrogen in the digestive tract is not affected by lack of *B* that the secretions of the glands associated with digestion and absorption are in no way dependent upon the supply of vitamins.

Uhlmann [98] found that the administration of vitamins to cats and dogs, either subcutaneously, intravenously, or by the mouth, caused a marked stimulation of the salivary, sweat, gastric, and other glands, which is inhibited by atropine. From these observations he concluded that the vitamins in the food play an essential part in the

[96] Lumiere, Bull. acad. med. Paris, 83, 310; 84, 274, 1920; Par. Med. 10, 474, 1920; J. Am. Med. Assoc. 74, 1607, 1920.

[97] Karr, J. Biol. Chem. 44, 277, 1920.

[98] Uhlmann, Zeitsch. Biol. 1918, 68, 419-456.

processes of digestion and metabolism through their stimulatory action on glands producing external and internal secretions.

The work of **Funk and Douglas, McCarrison,** and **Emmett and Allen,** showing the marked degenerative changes in the glands of internal secretion, appear to indicate a very definite relation between these glands and the vitamins, and this is further suggested by **Swoboda's** [99] findings that *B* is present in large quantities in most of the organs of internal secretion which are of developmental importance, but in much lower concentrations in other organs, with the exception of the liver and kidney. **Hopkins** [100] has given it as his opinion that there is increasing probability that the function of the accessory food factors is connected with the stimulating of the internal secretions. In the light of our constantly increasing knowledge of the inter-relation between the internal secretions and their immense importance in metabolism, the dependence of these glands upon the vitamins, could such be proved, would be entirely sufficient to account for all the numerous symptoms which have been noted as resulting from vitamin deficiency. Accepting this hypothesis provisionally we are led to inquire whether there is anything in the nature of the vitamins which might explain their effect upon the secretory organs.

There are two classes of factors which play a well-recognized part in physiological processes, the hormones and the enzymes. In certain respects, such as the difficulty met with in attempting to separate and identify chemical individuals and notable sensitiveness towards heat and reagents, the vitamins show a resemblance to the enzymes. Moreover, the predominance of enzymic activity in connection with physiological processes inclines us to ascribe all otherwise inexplicable results to enzyme action. We have, however, no definite evidence that the action of the vitamins is catalytic. On the other hand, **Delf's** [101] observation that the rate of destruction of the antiscorbutic vitamin in cabbage leaves is increased only about three-fold for an increase of 30° to 40° in temperature would indicate that here at any rate we are not dealing with an enzyme-like body.

The term hormone, meaning a substance which arouses or excites, was used by **Starling** for certain substances which have the power of arousing functional activity in the animal organism, apparently by stimulating enzyme activity in the cells to which they gain entrance. To this class belong such substances as secretin, the excitant

[99] **Swoboda, J. Biol. Chem. 44, 539, 1920.**

[100] **Hopkins, Roy. Soc. Med. [Sect. Ther. & Pharm.] Lanc. 1919, ii, 979.**

[101] **Delf, Bioch. J. 12, 416, 1918.**

of the pancreatic gland, and gastrin, the hormone which induces the secretion of the gastric juice.[102]

The classification is based on function entirely and is in no way connected with chemical nature. **Armstrong**[103] supposes that the metabolism of plants as well as animals is determined by hormone and suggests that the substances associated with glucose in glucosides for instance may function as hormones, the glucoside being hydrolyzed during germination and providing a hormone which serves to stimulate the growth of the seedling.

It has been suggested that the vitamins are hormones which stimulate the endocrinic glands to produce their secretions, which in turn stimulate the various cells of the body. **Mendel** first used the term hormone in connection with vitamins,[104] and **Hopkins** afterwards adopted the term "exogenous hormones." **Voegtlin** and **Myers**[105] have pointed out the close similarity, if not identity, of the antineuritic vitamin from yeast with pancreatic secretin. They have succeeded in relieving to some extent at least the neuritic symptoms induced in animals by deficient diets by the administration of secretin, while on the other hand, when injected into dogs, the antineuritic vitamin stimulates the flow of bile and pancreatic juice. A yeast preparation which had lost its curative power for avian polyneuritis was likewise devoid of any stimulating effect on pancreatic secretion or flow of bile.

McCollum[106] objects to this view. "Food hormones is an objectionable term because all the evidence available indicates that both the fat-soluble *A* and water-soluble *B* are never-failing constituents of the cells of both animal and plant tissues. They have nothing in common with the hormones. The latter are chemical substances which are formed in the body by special tissues and contributed to the blood stream where they cause the stimulation of certain other tissues to physiological activity. They are chemical messengers, while the substances under discussion are food complexes apparently necessary for all the living cells of the body."

There seems no reason, however, for thus limiting the conception of hormone. **Mathews**[107] finds some basis for thinking that hor-

[102] **Edkins**, J. Physiol. 34, 132, 1906; **Keeton** and **Koch**, Am. J. Physiol. 37, 481, 1915.

[103] **H. E.** and **E. F. Armstrong**, Ann. Bot. 25, 507.

[104] See **Lusk**, Sci. Nut. 1917, 378.

[105] **Voegtlin** and **Myers**, Am. J. Physiol. 49, 124, 1919; J. Pharm. Exp. Ther. 13, 301, 1919.

[106] **McCollum**, Newer Knowledge of Nutrition, p. 85.

[107] **Mathews**, Physiol. Chem. 1915, 448.

mones are normally produced perhaps in all cells under the influence of the nerves, and that these substances directly stimulate the cell to activity. From this point of view there could be no objection to regarding the vitamins either as hormones or as precursors of the hormones of the endocrinic glands. The improved condition noted in pigeons after administration of *B* and the apparent increased metabolic rate noted in deficiency studies when the lacking essential is supplied, according to **Dutcher,** are due to increased activity of the glands of the alimentary tract and the organs of internal secretion, with the result that foods are more completely digested and assimilated, and the stimulation of cell activity due to hormone action is increased.[108] This investigator has endeavored to relate the antineuritic vitamin with oxidative processes. He found that the catalase content of tissues of pigeons on a diet of polished rice falls until it reaches its lowest level at the acute stage of polyneuritis, a result which is in accord with **Ramoino's**[109] observation that the respiratory quotient falls in avian polyneuritis, but when extracts of rice bran are added and the pigeons have started to recover the respiratory quotient increases to normal. On administration of antineuritic *B* the catalase likewise tended to return to normal. **Dutcher** believes that the vitamin does not itself act as a direct activator of catalase but stimulates the organism to greater production of this enzyme. In this connection **Bachman's**[110] statement that vitamins are essential for the growth of anaerobic yeasts while an aerobic yeast grew readily in a synthetic medium without the addition of vitamin is interesting. **Findlay**[110a] has recently confirmed Dutcher's observation that the glyoxalase content of the liver is less in pigeons with beriberi than in the control birds and concludes that the vitamin does not act as a co-enzyme of glyoxalase.

Tschirch's theory of the biological function of vitamins[111] in building up the animal organism, is as follows: The organism cannot, unaided, synthesize from aliphatic amino acids such cyclo compounds as, e. g., purine and pyrimidine bases, which are so essential in the formation of nucleic acids, or cellular nuclei. The synthesis, however, takes place in presence of enzyme-like substances, "Cyclokeiases," i. e., "ring closers," which contain, or perhaps are identical with the vitamins themselves.[112]

[108] **Dutcher,** J. Biol. Chem. 36, 63, 547, 1919.

[109] **Ramoino,** Arch. Ital. biol. 65, 1, 1916-17.

[110] **Bachman,** J. Biol. Chem. 39, 235, 1919.

[111] **Schweiz,** Med. Wochschr. No. 2, 21, 1920.

[112] **Haussler,** Schweiz, Apoth.-Ztg. 58, 621, 634, 655, 1920; Chem. Abs. March, 1921, 701.

McCarrison's noteworthy summary of the functions of vitamins [113] very concisely and positively sets forth their bearing on health and disease:

"(1) Vitamines are constant constituents of living tissues. Although present in very small amounts, maintenance of health is dependent on their action.

"(2) Vitamines do not themselves contribute to the energy supply of the body but facilitate utilization by it of proteins, fats, carbohydrates and salts of food.

"(3) Proteins, fats, carbohydrates and salts cannot support life without vitamines, nor vitamines without these proximate principles; they are complementary to each other. Without vitamines, the body starves.

"(4) A distinct relationship exists between the amount of vitamines required and the balance of food in protein, fat, carbohydrate and salt, the efficacy of the vitamine depending on the composition of the food mixture.

"(5) A distinct relation exists between the amount of vitamine required and the rate of metabolic process.

"(6) Each vitamine plays a specific part in nutrition.

"(7) It appears that vitamine *A* is associated with the metabolism of lipoids and calcium, as well as with chemical reactions requisite for growth and maintenance.

"(8) Vitamine *B* appears to be associated with the metabolism of carbohydrates and with the chemical reactions and functional perfection of all cells, particularly nerve cells.

"(9) Vitamine *C* appears to be associated with the metabolism of calcium and with the chemical reactions of growing tissues.

"(10) All vitamines are concerned in the maintenance of orderly balance between destructive and constructive cellular processes.

"(11) One vitamine cannot replace another although its function may be interfered with by the absence of another.

"(12) The final result of their efficiency is the same whatever be the degree of deprivation, the greater the deprivation the more rapid is the onset of symptoms due to it, the lesser the deprivation the slower is the onset of the symptoms due to it.

"(13) Each vitamine exercises a specific influence on the adrenal glands; the effect of their deprivation on these organs is one of the most outstanding features of deficiency diseases.

"(14) Vitamines influence markedly the production of hormones and all external secretions.

"(15) There is reason to believe that the capacity of any given cell for work is impaired in proportion to the degree of vitamine starvation.

"(16) Vitamines aid the tissues in resisting infection.

"(17) Vitamine, especially vitamine *B*, induce in the human and animal body a desire for food.

"(18) Vitamines are one link in the chain of essential substances requisite for harmonious regulation of chemical processes of healthy cellular action. If the

[113] Studies in Deficiency Diseases, Oxford University Press, 1921, Am. Food Jour. Oct. 1921.

link be broken, harmony ceases or becomes discord as it may cease or become discord if any other link be broken.

"(19) The place of vitamines in human economy must be considered in connection with metabolism as a whole: in connection with their relation to other essential food requisites, with their relation to organ digestion and assimilation, and with their relation to endocrine regulators of metabolic processes."

CHAPTER X

RICKETS

Findlay, in his admirable historical survey of rickets,[1] defines this disease as a derangement of which the chief manifestation is to be seen in the growing bone, in which there is an excessive and irregular development in its earlier stages, with arrest or delay in the formation of fully-formed, firm and normally calcified bone.

As long ago as 1650 the symptoms were described with accuracy by **Glisson**,[2] of whose description **Findlay** makes the comment that "there has been nothing to withdraw and very little to add." With regard to the external appearance of a rachitic child **Glisson** states:

> The parts of the child are irregular and of unusual proportion. The head is larger than normal, and the face fatter in respect to the other parts. The external members and muscles of the body are seen to be delicate and emaciated. The whole skin, both the true and the fleshy and fatty layers, is flaccid and rather pendulous, like a loose glove, so that you think it could hold much more flesh. The joints are not firm or rigid. The chest externally is thin and much narrowed.

The disease is further characterized by restlessness and perspiration at night, and occasionally by sensitiveness of the limbs so that the child cries on being handled. Many writers describe gastro-intestinal disturbances as characteristic of rickets, but **Findlay** states that in his experience normal digestion is the rule rather than the exception, the characteristic protruding abdomen being due partly to degenerative changes in the walls of the stomach and intestines and partly to the abnormally small size of the thoracic cavity. It may be noted in this connection that scurvy and rickets are not infrequently found in conjunction and the symptoms of the one may very easily have been occasionally confused with symptoms of the other.

The ends of the long bones are enlarged, especially at the wrists and ankles, and the bones themselves are variously distorted. A curious feature of the disease is the fact that the various bones seem to be unequally affected in different cases. In one case the legs may suffer chiefly, in another the arms, in still another the thorax, and in

[1] Med. Res. Com. Sp. Rep. 20.

[2] **Glisson**, De rachitide, London, 1650.

others again the head; nevertheless, in not a few cases, all parts of the skeleton are equally and severely affected.[3] Very characteristic is the "beading" of the ribs, due to the development of nodules at the juncture of the ribs with the costal cartilages, which if very prominent may be seen as a string of beads down each side forming the "rickety rosary."

Analysis shows that the rachitic bone is strikingly deficient in calcium. **Langstein** and **Meyer**[4] give the following results for the analysis of a rachitic and a normal bone:

	Normal Radius. Per cent	Rachitic Radius. Per cent
Cartilaginous material	29.43	71.26
Salts of lime	66.33	18.26

Owing to this deficiency the rachitic bones are soft so that they can readily be cut with a knife and bend easily. The following clear description of the bony structure is taken from **Findlay** (l. c.):

Macroscopic characters.—On section of any long bone the most striking abnormality is the increase in width of the cartilaginous growing zone. Normally, this growing zone is truly a line—the epiphyseal line—forming a narrow, glistening, bluish-white band, sharply demarcating the diaphysis from the epiphysis. In rickets it is increased in thickness many fold, is irregular, and extends by tongue-like processes into the diaphysis. Sometimes these areas of cartilage are completely cut off from the growing epiphyseal zone and, as suggested by **Virchow,** may form nuclei for the development later of enchondromata. Occasionally this hyperplastic tissue retains the appearance of having been deposited in layers, the one layer being separated from the other by a vascular zone. More frequently, however, no such arrangement is visible, and irregular islands of uncalcified cartilage separated by vascular spaces predominate. These vascular areas give the idea of hyperaemia, and have suggested the view that the condition is inflammatory in nature. The narrow cavity seems unduly wide, and there is the appearance of increased bony tissue under the periosteum.

Microscopic characters.—On microscopic examination the irregularity of the epiphyseal zone is even more striking.

At the growing end of the shaft of any long bone of the normal child the first change observed is a proliferation of the cartilage cells, which subsequently become arranged in rows in the long axis of the bone. The cell spaces enlarge and become confluent, forming the primary narrow spaces. The cartilage cells then degenerate, and the spaces are invaded by vessels from the perichondrium, accompanied by osteoblasts and connective tissue. The vessels of the perichondrium encircle the bone in rows as hoops do a barrel, and thus the invading vessels are arranged at different levels and give to the bone a somewhat striated

[3] **Findlay,** l. c.

[4] **Langstein** and **Meyer,** Sauglingsernahrung and Sauglinsstoffwechsel. Wiesbaden.

appearance.[5] These branches enter the bone at right angles to the long axis, and ramify in the primary marrow spaces; they do not anastomose, but terminate as end arteries. According to **Schmidt**, these vessels vary in number in different bones, and their relative abundance is a measure of the rate of growth of the bone. The cartilaginous matrix between the cells next becomes calcified, and these pillars of calcified cartilage form a scaffold on which bone is deposited. The normal body trabeculae thus formed are occupied for the most part by almost parallel rows of elongated bone cells, with their processes running at right angles to the margin, and are surrounded by a mere rind of homogeneous "osteoid" tissue.

In rickets there is an excessive preparation for the formation of bone in the greatly increased depth of the layer of proliferating parallel-arranged cartilage cells. The disappearance of the cells is delayed, and occurs erratically, so that irregular areas of cartilage remain as such, and are neither absorbed nor infiltrated with lime. These tongue-like processes of cartilage alternate with areas of partially or almost normally calcified tissue, with an apparent increase of the intervening marrow. When ossification occurs it takes place irregularly. The trabeculae persist for the most part, however, as homogeneous processes with or without ghosts of cartilage cells — the so-called osteoid tissue — with small central zones occupied by dwarfed and irregularly placed bone cells.

It is the development of this osteoid tissue that is the true characteristic histological feature of rickets.

This disease appears to be remarkably wide-spread. **Schmorl**[6] states that histological investigation on 386 children dying before the age of four years showed that 90 per cent had rickets. **Dick**[7] has reported that 80 per cent of the children in the London County Council schools were found to have symptoms of this disease, and **Findlay** (l. c.) states that evidence points to an incidence of probably not less than 50 per cent in the general population of the industrial areas of Great Britain. **Hess** found that 90 per cent of the children in a New York orphan asylum were affected. In a recent paper on rickets in Germany, **Engel**[8] asserts that the number of rachitic children constantly increased during the war. These children in many cases were dwarfed, deformed, and in a number of cases unable to walk at the age of three, four and even six or seven years. It is estimated that in the town of Dortmund, where there were about 30,000 children, 10 per cent cannot walk. **Dalyell**[9] reports from Vienna that in one community, which included many breast-fed infants, rickets was diagnosed in 50 per cent of the children of five months and 100 per cent of those of nine months of age.

[5] **Schmidt and Schmorl**, Ergeb. d. enn. Med. u. Kind. 4, 403, 1909.
[6] **Schmorl**, Verhand, der. deutsch. path. Gesel. 1909, 58.
[7] **Dick**, Proc. Roy. Soc. Med. Sect. for Dis. Child., 1915, p. 83.
[8] **Engel**, Lanc. 1920, i, 188.
[9] **Dalyell**, Brit. Med. J. July 31, 1920.

Many theories have been advanced to account for the development of rickets. Some of these are now generally discredited and may be dismissed with a word.

Heredity appears to have no direct connection, although **Siegert**[10] believes that the appearance of the disease in breast-fed children may be accounted for on this ground.

The view common at one time that rickets was to be regarded as a manifestation or result of congenital syphilis no longer receives any consideration.

The theory of an infective organism has been held by various authors,[11] some of whom claim that they have induced the disease by inoculation or contact with an infected animal. Recent work has not confirmed this view, however, and it seems probable that the observed results are susceptible of other interpretation.

While some writers have maintained that want of breast feeding is the cause of the disease, others have held that too prolonged suckling is responsible. The work of **Findlay**[12] has eliminated both these possibilities.[13]

Attempts to establish a direct connection between rickets and deficiency of some internal secretion have been equally unsuccessful.[14]

Faulty diet or digestion resulting in the production of acidosis of some form of intoxication has been suggested by several writers as the cause of the rachitic condition, but this has never been confirmed and, on the other hand, other forms of acidosis produce no such decalcification of the bones. **Lienaux and Huynen**[15] regard both rickets and osteolmalacia as a special type of acidosis in the broader sense, due to the unsatisfactory proportions of the calcium and the phosphoric acid in the diet. They base their theory on observations made on farm animals and the satisfactory results obtained by addition of lime to a calcium-poor diet. It has been found, however, that abundance of calcium alone, whether supplied in the form of milk or as calcium phosphate, will not necessarily protect against rickets, while

[10] **Siegert**, Verhand. d. Gesell. f. Khde. 1903; Jahrb. f. Khde. 58 and 59.

[11] **Torane** and **Salvatore Forte**, La Pediatria, Sept. 1907; **Moussu**, Bulletin de la Societé Centr., Vol. LVII.; **Koch**, Zeit. f. Hyg. und Infektions-krankheiten, 72, H. 2, 321; **Koch**, Zeit. f. Hyg. und Infektons-krankheiten, 69, 436; **Mircoli**, Pathologica, 3, 562 and 566, 1911; **Marfan**, Le Rachitisme et sa Pathogenie, Paris, 1911; **Koch**, Arch. wiss. prakt. Tierheilkunde, 45, 263, 1919.

[12] **Findlay**, Lanc. May 8th, 1915.

[13] See also **Davidsohn**, Ztsch. f. Kinderh. 21, 349, 1919.

[14] **Stoeltzner**, Jahrb. f. Khkde, 51 and 53; **Basch**, *Ib*. 64, 289, 1906; **Matti**, Ergeb. d. inn. Med. u. Kind. 10, 1, 1913; **Klose** and **Vogt**, Beitr. z. klin. Chir. 79, 1910; **Renton** and **Robertson**, J. Path. Bact. 21, 1916.

[15] **Lienaux** and **Huynen**, Bull. acad. roy. med. Belg. 29, 855, 1919.

a diet low in calcium may not produce rickets even though evidence of the calcium deficiency is found in the softness of the bones.[16]

Several distinguished authorities [17] have favored the hypothesis that rickets arises from deficiency of fat in the diet,[17a] a view which received support from the often-quoted work of **Bland-Sutton** on lion cubs in the zoölogical gardens,[18] in which the addition of cod-liver oil, milk, and crushed bones to the ordinary diet of lean meat was found to protect the animals against rickets, and also from the well-recognized beneficial effect of cod-liver oil in the treatment of rickets.[19] That the fat in itself is not the fundamental factor is clearly proved by the work of **Mellanby,**[20] who has shown that different fats have very different values in this respect; of **Osborne** and **Mendel** [21] and **von Groer,**[22] who have shown that normal nutrition can be secured for a considerable time, at least, on diets almost devoid of fat; of **Mackay** and **Tozer** [22a] who have studied the effect on kittens of a diet deficient in fats, and found no evidence of rickets, although the kittens became emaciated, ceased growing, and suffered from diarrhoea and abdominal

[16] **Stoeltzner,** Jahrb. f. Kinderh. 50, 268, 1899.

[17] **Holt,** Dis. of Inf. and Child. 1907; **Cheadle,** Artific. Feeding and Food Disorders of Infants, 1906.

[17a] As a result of feeding experiments and clinical reports, it has been accepted that vitamin *A* is highly important for man, and that the lack of it leads to nutritional disorder in children. To study this question, five infants, ranging in age from 5 to 12 months, were given a diet complete except for a very small amount of vitamin *A*. It consisted of 180 g. daily of skimmed milk (0.2% fat), 30 g. cane sugar, 15 to 30 g. autolized yeast, 15 c. c. orange juice, 30 g. cottonseed oil, and cereal for the older infants. On this diet no detrimental effect was observed over a period of 8 to 9 months. These results suggest that either a very small amount of this vitamin suffices to supply the needs of human nutrition, or that this deficiency has to be maintained for a period of years to bring about harmful result. Infants fed on this diet did not develop signs of rickets. The disorder developed however in infants receiving large quantities of milk containing ample vitamin *A*. **Hess** and **Unger,**[17b] therefore, do not believe that rickets is brought about by the lack of this principle.

Golding, Drummond and **Howard** (Biochem, J. 15, 427, 1921) carried out experiments on rationing pigs with extracted dried milk, cream, purified caseinogen and salt mixture. At the same time, feeding tests of a like nature were carried out except that autoclaved olive oil was substituted for cream. The results obtained do not offer any definite information on the relationship of fat-soluble *A* to the etiology of rickets.

[17b] Proc. Soc. Exptl. Biol. Med. 17, 49, 1919.

[18] Quoted by **Cheadle** and **Paynton, Allbut's** Syst. of Med. 3, 85.

[19] **Hess and Unger,** J. Am. Med. Assn. 1917, 1583.

[20] **Mellanby,** Lanc. 1919, i, 407.

[21] **Osborne** and **Mendel,** J. Biol. Chem. 45, 145, 1920.

[22] **Von Groer,** Bioch. Z. 97. 311, 1919.

[22a] **Mackay** and **Tozer,** Biochem. J. 15, 19, 28, 1921.

distension; and of **Shipley, Park, McCollum, Simmonds,** and **Parsons,**[23] who have demonstrated that cod-liver oil contains some substance or substances which cause the deposition of calcium in the bones in the same way in which it occurs in spontaneous healing of rachitis in man. However this is disputed by Telfer (see reference on p. 177).

Defective hygienic conditions of various sorts, bad ventilation,[24] insufficient lighting,[25] and lack of exercise [26] have all been represented as responsible, and **Hansemann** [27] has classed together all the unwholesome features of civilization under the comprehensive term " domestication " and regards the disease as the product of the combination. In this connection the observation of **Hess** and **Unger** [27a] that exposure to sunlight had a marked and curative effect on rickety children, is of interest.

Ferguson's statistical study of the social and economic factors in the causation of rickets [28] indicates inadequate air and exercise as potent factors in determining the onset of rickets, and minimizes the effect of diet. In this study the average number of persons per room was found to be almost one person greater in the rachitic than in the non-rachitic families, the cubic space per person was 32 per cent less in families with cases of marked rickets than in families who were free from the disease, and the cleanliness of the house was distinctly better in the non-rachitic than in the rachitic family. Commenting on this investigation, **Mellanby** [29] says:

> In the study of rickets in Glasgow made by **Ferguson,** analysis of the average diets of rachitic and non-rachitic families studied shows that in practically every case the diet of the rachitic families contained more of substances allowing rickets such as flour, potatoes, sugar, oatmeal, and less of antirachitic foods, such as milk, meat, fat, fish, eggs, and cheese. While the differences are not large, they probably indicate a very decided dietetic disadvantage to the children of the rachitic families.

[23] **Shipley, Park, McCollum, Simmonds** and **Parsons,** J. Biol. Chem. 45, 343, 1920.

[24] **Dudgeon,** Rep. of the Soc. for Study of Dis. in Child, Vol. VIII, p. 56; **Clement Lucas,** Brit. Journ. of Child. Dis., Feb. 1908; **Esser,** Münch. Med. Woch. 818, 1907; **Kassowitz,** Die Pathogenese der Rachitis. Wien. 1885.

[25] **Diesing,** Deutsch. Med. Wochschr. 39, 552.

[26] **Findlay,** Brit. Med. J. 1908, ii. 13; **Paton, Findlay,** and **Watson,** *Ib.* 1918, ii. 625.

[27] **Hansemann,** Berl. klin. Woch. 1906, Nos. 20 and 21.

[27a] **Hess** and **Unger,** J. Am. Med. Assoc. 77, 39, 1921.

[28] Med. Res. Com. Spec. Rep. 20.

[29] **Mellanby,** Lanc. 1920, i. 856.

While admitting that poor surroundings and lack of exercise may be powerful predisposing factors, **Mellanby** refuses to consider these as direct causes. He points out that the diets used by **Findlay** (l. c.) were such as will eventually produce rickets regardless of other conditions.

The beneficial effect of freedom, he states, is what might be expected, as constant movement must raise the whole metabolic changes in the body and in the first place prevent or delay deposition of fat with its accessory *A*, and secondly bring into activity any antirachitic factor normally stored away and ineffective. Exercise therefore gives greater opportunity for any antirachitic factor in food or tissues of animals to play its part in the animal economy.

In a very extensive investigation involving the use of over 200 puppies [30] **Mellanby** believes he has obtained conclusive evidence that rickets is caused by the lack of a specific antirachitic factor which is either identical with or similar to the vitamin *A*. In his experimental work he made use of four types of rachitic diets.

I. Whole milk, 175 cc., oatmeal, rice; salt, 1–2 g.
II. " " " " bread, ad lib.
III. Separator milk, 175 cc.; bread (70 per cent wheat) ad lib; linseed oil, 10 cc.; yeast, 10 g.; salt, 1-2 g.
IV. Separator milk, 250 to 350 cc.; bread (70 per cent wheat), ad lib.; linseed oil, 5 to 15 cc.; yeast, 5 to 10 g.; orange juice, 3 cc.; salt, 1-2 g.

Using diet I, he found that increasing the whole milk from 175 to 500 cc. per day prevents the development of rickets.

On diet II, meat, and also watery and alcoholic (80 per cent) extract of meat, had an inhibiting effect. On the other hand, the protein residue after loss of the extractives allows rickets to develop. Yeast extract or yeast had no protective influence. Malt extract had some inhibiting action and delayed the onset of rickets when added to diet II.

A large number of animal and vegetable fats and oils were tested, and it was found that all were preventive except linseed.[30a] When milk

[30] **Mellanby,** Lanc. 1919, i. 407; 1920, i. 856.

[30a] A diet given to kittens to determine the effect of a deficiency of animal fat (**Mackay,** Biochem. J. 15, 19–27, 1921) consisted of white bread ad lib., orange juice 1 c. c., alternate days; marmite (a variety of yeast) 2 g. given in milk, whole not consumed. In addition, control animals received whole milk ad. lib., and the experimental animals machine-skimmed milk with 3% olive oil ad. lib. Both the whole and skimmed milk were brought to a boil and allowed to cool before being given to the kittens. The fat value of the skimmed milk was 0.15%. The olive oil had previously been autoclaved, and had been shown to be free of *A*. The absence of *A* in the diet of the experimental animals gave rise to emaciation, arrest of growth, abdominal distension with atrophy of the walls of the stomach and intestines in some cases and changes in the costo-

fat is eliminated from the diet by using separator milk, and linseed oil was the only fat in the basal diet, it was found that the value of the oils is graded, cod-liver oil being the best and linseed oil worst. Animal fats are more antirachitic than vegetable fats, and the latter differ among themselves. The best vegetable fats are peanut and olive, the least effective of those examined include linseed, cottonseed, babassu oil, a hydrogenated fat, and coconut oil. From this it would seem that if *A* and the antirachitic factor is identical we must assume that development or lack of development of rickets in puppies is a much more delicate test for the presence of *A* than are growth experiments with rats.

It was found that the addition of orange juice (one-fourth of an orange per day) did not prevent rickets, also that adding 5 g. of calcium phosphate or increasing the calcium intake by doubling the amount of separator milk, had no preventive effect, but that under these conditions the growth and general health of the puppies was better, and in general the better an animal grows on a rachitic diet the more easily is rickets produced.

On diets III and IV it was found that small quantities of meat and meat extracts did not prevent rickets from developing as had previously been observed when used in addition to diet II. Although meat did not prevent rickets, however, it was found to have some inhibitory effect, which was best seen when small quantities of meat were given with quantities or types of fat otherwise ineffective in preventing rickets. This probably accounts for its action on diet II, where a small amount of butter-fat in the milk had its antirachitic effect enhanced by meat. In this connection **Mellanby** remarks:

> Meat evidently has a stimulating action on growth far beyond its *A* content, and the antirachitic action is also greater than would be expected from the amount of *A* supposed to be present. It is known that meat, more than any other foodstuff, has a stimulating effect on the total chemical changes taking place in the body. In thus serving as a stimulant, it will increase the effectiveness of any *A* in the diet, and will tend to prevent storing up and deposition of this substance in subcutaneous and other tissues. Also, any *A* in the tissues will be more readily metabolized under the stimulating influence of the metabolizing meat. It is probable that the antirachitic action of meat may therefore be due more to making the fires burn more brightly and thereby increasing the effectiveness of any *A* present in the body rather than to the amount of this factor which it possesses in itself.

---

chondral junctions similar to those seen in guinea pigs and rats suffering from deficiency of the same accessory food factor. The appearance presented by these animals was similar to the clinical picture of celiac disease in children. No evidence of rickets was found in any of the kittens. (See also **Tozer**, Biochem. J. 15, 28, 1921).

Substances found to have *no preventive action* against rickets are: Separator milk, bread, protein of meat, yeast, linseed and babassu oils, and certain hydrogenated fats.

Substances with *well marked preventive action:* Cod-liver oil, butter, suet.

Substances with *definite, but slighter, preventive action:* Meat, meat extract, malt extract, lard, peanut, and olive oil.

In his later paper **Mellanby** emphasizes the importance of the mutual relation of all the dietetic elements.

Any condition which leads to laying on of tissue seems to necessitate a greater intake of antirachitic accessory factor to prevent rickets, while conditions which stimulate metabolism and increase the loss of heat relatively to the energy of the stored food, work together with the antirachitic accessory factor, and reduce the amount necessary for normal growth. Excessive carbohydrate in the diet often leads to obesity and lethargy; confinement works in the same direction, and the diet under these conditions must have more than the normal amount of antirachitic accessory factor to prevent the development of rickets. Proteins and exercise, on the other hand, are stimulants to metabolism, and when the diet has a relatively high protein content and the animals are active less antirachitic accessory factor is necessary.

Hess [31] reports a case of five infants varying in age from five to twelve months who were given a diet which was complete except for a very small content of fat-soluble vitamin. They thrived on this diet for eight to nine months and showed none of the well-established signs of rickets. From this, **Hess** concludes that either a very small amount of this vitamin suffices to supply the needs of human nutrition or this deficiency has to be maintained for a period of years in order to bring about any harmful result. **Hess** does not believe that rickets is brought about merely by a deficiency of *A*, as this disorder developed in infants receiving large quantities of milk containing an ample supply of this vitamin. Moreover, neither cream nor the leafy vegetables, both of which are rich in *A*, are regarded as comparable to cod-liver oil as antirachitics.[31a]

[31] **Hess,** Proc. Am. Soc. Biol. Chem., J. Biol. Chem. 41, xxxii. 1920; J. Am. Med. Ass. 76, 693, 1921.

[31a] **Hess** and **Unger** (Proc. Soc. Exptl. Biol. Med. 17, 220, 1920) also finds that many diets supposed to be conducive to rickets result in normal nutrition of infants. Condensed milk only exceptionally induced rickets. A "protein milk" made by precipitating buttermilk with heat regularly led to rickets. It contained 0.44 per cent ash, in which Ca and P stood midway between that of human and cow milk; its Na content was even higher than in cow milk. Its

That the curative power of cod-liver oil for rickets [32] is not associated with its content of *A* is indicated by the work of **Phemister, Miller** and **Bonar,**[33] who found that phosphorus and cod-liver oil and phosphorus alone had quite similar results. While it is not possible to make a definite statement without further investigation, the results so far obtained suggest that phosphorus may be as effective as the cod-liver oil alone.

McCollum and his associates [34] have published a preliminary communication on the production of skeletal derangements in rats by deficient diets. Certain of the pathological changes observed are identical with those seen in the bones of rachitic children, while others are similar to but not identical with these. They have proved that these conditions are undoubtedly due to the diet alone, but hesitate to ascribe them to one specific factor in the diet. From the observed results a low content of *A*, low calcium content, poor quality of proteins, and unsatisfactory salt combinations, acting together may all be contributory causes, and as the condition can be induced in several different ways it is suggested that there may be more than one kind of rickets.

Mackay [35] has carried on a clinical investigation of fifty-one rachitic children, the results of which, while not entirely accurate on account of the conditions under which the work had to be carried on (through the outpatient department of the hospital) are in accordance with the view that rickets is a dietetic disease, commonly developing in children receiving insufficient fat and too much carbohydrate, especially when bottle-fed.

An important contribution to this subject has been made recently by **Paton** and **Watson.**[36] These experimenters found that a liberal allowance of milk fat, up to even 14 grams per kg. of body-weight does not prevent the onset of rickets in young dogs under ordinary laboratory conditions, whereas pups kept largely in the open air may escape the development of rickets on an intake of less than 1 gram of milk fat per kg. of body weight. With scrupulous care as to cleanliness

---

fat-soluble vitamin content was high, the water-soluble vitamin low.

The factor which plays a rôle in the etiology of rickets is possibly a fourth unidentified dietary factor or vitamin. (**McCollum,** Dental Cosmos, 63, 596, 1921.)

[32] **Howland** and **Park,** Tr. Am. Pediat. Soc. 1920.

[33] **Phemister, Miller,** and **Bonar,** J. Am. Med. Ass. 76, 850, 1921.

[34] **McCollum, Simmonds, Parsons, Shipley** and **Park,** J. Biol. Chem. 45, 333, 1920.

[35] **Mackay,** Brit. Med. J. 1920, ii, 929.

[36] **Paton** and **Watson,** Brit. J. Exptl. Pathol. 2, 75–94, 1921.

it is, at least sometimes, possible to rear pups free of rickets in the laboratory on an intake of only about 0.5 grams of milk fat per kg. of body weight, along with bread, provided that the diet affords an adequate supply of energy. The energy value of the diet would seem to play a part in controlling the development of rickets, but that it is only a contributory part is shown by the development of rickets in pups with a high energy intake if confined without scrupulous care as to cleanliness. Milk fat may be reduced to about 0.3 grams per kg. of body weight, if its place is taken by an equal amount of lard, without the onset of rickets. In view of these observations it seems necessary to conclude that both environment and diet are important factors in the development of rickets, sometimes one, and sometimes the other, predominating in a particular case.

CHAPTER XI

BERIBERI

THE disease of beriberi has been common in the Orient for many years. The Malay States, Siam, Corea, parts of Japan, and the Philippines have all been extensively afflicted with it. **Holst**[1] reports that of the ships arriving at Falmouth in the year 1900 from harbors south of 33° North, 0.5 per cent of the English ships and nine per cent of the Norwegian ships had cases of beriberi on board. Reports of the disease have come from India, Africa,[2] Marseilles,[3] and Spain.[4] According to **Fraga**,[5] epidemics have frequently been reported in Brazil, although often under other names. The first serious outbreak in North America was reported by **Little** in Newfoundland and Labrador in 1912,[6] but occasional cases have been reported from time to time in the South and West of the United States.[7]

Simpson[8] has reported some interesting observations which seem to indicate that natives of certain localities may show a greater predisposition than others to this disease. Of different nationalities admitted to the beriberi hospital in Singapore, Chinese were six times as numerous as all others.[9] While the disease in its true form is of common occurrence among recent immigrant Chinese, it is practically unknown among Straits-born settlers in the colony. The disease is most prevalent among the coolie class, those employed on rubber estates being especially prone to infection. Certain areas in Johore were constantly found to furnish the largest number of patients. During the same period, of a considerable number of sawmill coolies ad-

[1] **Holst**, Tr. Soc. Trop. Med. Hyg. 5, 76. See also **Holst** and **Frölich**, Z. f. Hyg. Infekt. 72, 1, 1912.

[2] **Wydooghe**, Bull. Soc. Path. Exot. March, 1918. Quoted by **Jelliffe**, J. Nerv. Ment. Dis. 49, 522, 1919.

[3] **Sicard, Roger**, and **Rimbaud**, Paris Med. Dec. 1, 1917.

[4] **Vinson**, Bull. Soc. Med. Maur. 1917.

[5] **Fraga**, New Orl. Med. Surg. J. 1918.

[6] **Little**, J. Am. Med. Ass. 58, 2029, 1912; 63, 1287, 1914.

[7] For further details as to the topographical incidence of beriberi see **Jelliffe**, l. c.

[8] **Simpson**, Lancet, 1919, ii, 1027.

[9] Japanese, Javanese, Malays, and Indians.

mitted from a wide area no less than three-quarters were found to have come from one mill. Of those employed at different trades, tailors alone accounted for more than half. Inquiries into former dietaries of a considerable number of beriberi patients, particularly as to the consumption of polished rice, showed no outstanding differences between them and that of the Straits Chinese, who did not appear to suffer from the disease in its true form.

In this connection reference may be made to the suggestion of **De Langen** and **Schut**,[10] that the liability of inhabitants of the tropics to certain diseases may be due to the excessively high content of sugar in the blood. A defective diet, they state, which in temperate climates would not be sufficiently deficient in vitamin to cause beriberi, might also in this way, in the tropics, induce that disease.

A high incidence of beriberi among women is reported in the Philippines, especially among mothers who are suckling babies. These women were found to be suffering from undernourishment, polished rice forming the greater part of their dietary. Their breast milk is poor in quality, to which is attributed the occurrence of beriberi in infants. **Andrews** induced several Filipino women whose infants had just died of beriberi to nurse young pups, and noted that in all cases the latter failed to grow, because edoematous, and lost the use of their hind legs.

Chemical analysis shows that human milk in beriberi differs very little in composition from the normal[11] but it is lacking in the antineuritic substance which is present in normal milk. **Hirota**[12] reports a few cases of beriberi in breast-fed infants whose mothers did not suffer from the disease, which can only be explained on the theory that through some abnormality the essential factor was not secreted by the mammary gland, although present in sufficient quantity for the normal metabolism of the mothers.

Beriberi was originally regarded as an infection, flourishing in insanitary surroundings, but in 1880 **Takaki**, Inspector General of the Japanese navy, carried out an investigation which discredited this theory and pointed to a purely dietetic origin. **Takaki** found that as far as sanitary conditions were concerned the ships of the Japanese navy which were ravaged by this disease compared favorably with the European ships which were entirely free from it. The only pronounced difference between the Japanese and the European hygiene

[10] **De Langen** and **Schut**, Geneesk. Tidjschr. v. Neder-Indie 56, 490, 1916. Trop. Dis. Bull. 9, 259, 1917.

[11] **Andrews**, Phil. J. Sci. 7B, 67.

[12] **Hirota**, Zent. f. inn. Med. 16, 385.

was to be found in the food, the Japanese ration at that time containing a much larger proportion of carbohydrate food, which was almost entirely in the form of polished rice. Just at this time a Japanese vessel had made a nine-months' cruise from Japan to New Zealand, Valparaiso, and Honolulu. Out of the 276 men on board 169 had developed beriberi, 25 cases being fatal. **Takaki** sent a second vessel over the same route with a similar crew and identical conditions except that a part of the rice ration was replaced by barley, vegetables, meat, and condensed milk. At the end of the cruise only 14 cases of beriberi had been reported, and these were all men who had refused to eat their allowance of the new foods. As a result of this test the character of the ration throughout the Japanese navy was gradually changed, in spite of the difficulty of educating the sailors to new dietary habits, with the gratifying result that beriberi almost disappeared. Whereas previous to 1884 nearly 33 per cent of the marines suffered from beriberi, during the six years after the adoption of the new diet, only 16 per cent were affected, and later the number of cases was reduced to two or three a year.[13] This beneficial effect was ascribed by the Japanese authorities to the increased amount of protein in the new diet, but strange to say neither the observed facts nor the explanation made much impression on medical opinion, according to which the disease was still to be regarded as an infection, despite the fact that all efforts to check it by improved sanitation proved unavailing.

In 1897, **Eijkmann,** a Dutch physician who was medical officer at a prison in Java, noted that a number of fowls which he was maintaining for experimental purposes had developed symptoms curiously like those of his human cases of beriberi. Careful observation showed that these symptoms were the result of an exclusive diet of polished rice, and that they disappeared on administration of rice polishings or substitution of hand-milled ("unpolished") for the machine-milled rice.[14] The name polyneuritis gallinarum was given by **Eijkmann** to the derangement as manifested in birds, and is still used to designate the avian disease, although practically all the latter researches tend to confirm the identity of this disease with human beriberi. **Fraga,**[15] however, dissents from this view, on the basis of an experiment in which a diet of polished rice, or polished rice plus either sterilized rice or sterilized beans was fed to nine convicts in solitary

[13] **Oshima,** Bull. 159, Office of Exp. Sta. U. S. Dept. Agric. 1905.

[14] **Eijkmann,** Arch. f. path. Anat. 148, 523; 149, 197, 1897; Arch. f. Hyg. 58, 150, 1906; Arch. Schiffs. u. Tropen-Hyg. 15, 699; 17, 328, 1913; Zentr. Bioch. Biophys. 12, 505.

[15] **Fraga,** Brazil, Med. 33, 49, 1919.

confinement, who volunteered for the experiment. The sterilized diet was distasteful to the subjects and was dropped at the thirty-sixth day on account of the onset of digestive disturbances, although no nervous disturbance developed. Another group remained on the diet for 43 days without the appearance of any symptoms. These rations produced typical polyneuritis gallinarum in fowls. **Fraga** concludes that avian polyneuritis and human beriberi are distinct morbid identities, but that dietary deficiencies act as a predisposing cause for beriberi in man.

Starting with the assumption that the polished rice diet was in some way responsible for the onset of the disease in fowls, **Eijkmann** investigated the diets in the Javanese prisons.

Taking the prison population at nearly 300,000, there was one case of beriberi per 10,000 among those living on unpolished rice, 416 per 10,000 among those living on a mixture of polished and unpolished rice and 3900 per 10,000 among those on polished rice. Experiments showed the missing element in the rice to be found in the layer of tissue under the husk known as silver skin, in the embryo, and in the bran. From these investigations, **Eijkmann** concluded that the substance which was removed neutralized another factor in the whole which was harmful.

Eijkmann's observations have been repeatedly confirmed both with fowls and with human subjects. **Fletcher**,[16] by experimenting with the diet in a lunatic asylum, demonstrated that when 28 ounces of rice was fed daily with only small amounts of other food the onset of the disease depended solely on whether the rice used was polished or unpolished. **Fraser** and **Stanton**[17] showed that the disease appeared among Japanese coolies after 80 to 90 days on a diet consisting mainly of polished rice, whereas a similar ration of unpolished rice appeared to confer immunity. **Grieg**[18] studied beriberi in India and also experimented with pigeons. He noted that those living mostly on polished rice and highly milled wheat showed much greater predisposition to the disease than those whose diet was more varied. Pigeons fed upon polished rice developed polyneuritis, while those fed on a mixture of wheat and pulses showed no symptoms of functional disturbances. **Couzien**[19] reported cases from Tonkin, which disappeared when the white rice in the diet was replaced by fresh hand-

[16] Quoted by **Sherman**, "Chemistry of Food and Nutrition," 2nd Ed. p. 320.

[17] **Fraser** and **Stanton**, Studies from Inst. for Med. Res. Fed. Malay States, Nos. 10 and 12; Lanc. 1909, 1, 451.

[18] **Grieg**, Sci. Mem. Med. San. Depts. India N. ser. 1911, No. 45.

[19] **Couzien**, Ann. Hyg. Med. colon. 15, 445; Zentral. Bioch. Biophys. 14, 909.

milled rice. **Cooper and Braddon**[20] demonstrated that the time of onset of polyneuritis in birds varied inversely with the quantity of polished rice fed to them.[21]

The prevalence of this disease among the Philippine scouts led to interesting dietetic experiments by **Chamberlain** and his associates.[22] When their investigation began the customary ration consisted of 12 ounces of fresh beef or its equivalent in bacon, canned meat or fish, 8 ounces white flour or bread, 8 ounces potatoes or onions, and 20 ounces of rice (polished). The only alteration made was the substitution of 16 ounces of unpolished rice and 1.6 ounces of dried beans for the 20 ounces of polished rice. **Chamberlain** states that the change to unpolished rice would have been unnecessary if they could have ensured the regular consumption of the bean ration by all the men, since even this small quantity of beans was quite sufficient to afford protection. In the course of two years after this change of diet the disease was practically eradicated.

Cox[23] reports that the Bureau of Science began to prepare extract of rice polishings in 1914, in which year 889 children under one year of age died of beriberi in Manila. Since then the amount of this extract used has steadily increased, with corresponding decrease in fatalities until now the only deaths from infantile beriberi recorded are a few cases in which illness was not reported to the health authorities until too late for treatment, as compared with the mortality of 95 per cent of all children attacked in 1914.

Evidence soon accumulated showing that rice was not the only food due to which beriberi might develop. **Vedder and Clark**[24] and **Ohler**[25] showed that typical polyneuritis developed in fowls fed on a diet of white bread or bread and rice.[26] **Little**[27] described out-

[20] **Cooper and Braddon**, J. Hyg. 14, 331, 1914.

[21] See also **Weill and Mouriquand**, Bull. de la. soc. ped. 16; J. Am. Med. Ass. 62, 1510; **Strong and Crowell**, Phil. J. Sci. 7B, 271; **Vedder**, *Ib*.. 415; **Funk**, Z. phys. Chem. 89, 373; **Fargier**, Ann. Hyg. Med. Col. 15, 491; **DeMello, Loundro, and Rebello**, Anals. Scient. da Fac. de Med. do Porto, 4, 6, 1917; **McCarrison**, Ind. J. Med. Res. 6, 275, 550, 1919; **Riddell, Smith, and Igaravidez**, J. Am. Med. Ass. Feb. 22, 1919.

[22] **Chamberlain**, Phil. J. Sci. 63, 133, 1911; J. Am. Med. Ass. 64, 1215, 1915; **Chamberlain and Vedder**, Phil. J. Sci. 6B, 25, 395; **Chamberlain, Bloombergh, and Kilbourne**, *Ib*. 6, 177; **Chamberlain, Vedder and Williams**, *Ib*. 7B, 39, 1911-12.

[23] **Cox**, Annl. Rep. Phil. Bur. Sci. 1918.

[24] Phil. J. Sci. 7, 423, 1912.

[25] **Ohler**, J. Med. Res. 31, 239, 1914.

[26] See also **Wellman and Bass**, "Polyneuritis gallinarum caused by Different Foodstuffs," Am. J. Trop. Dis. and Prev. Med. 1913, i. 129.

[27] **Little**, J. Am. Med. Ass. 58, 2029, 1912; 63, 1287, 1914.

breaks of the disease in Labrador and Newfoundland which he attributed to the diet of white bread, molasses, and fish upon which the inhabitants lived. While the only flour available was the product of the rough mills of the country, beriberi was unknown, but when the bolted flour of the modern mills became common, outbreaks of beriberi followed. It is told that in 1910 a ship ran ashore, laden with a cargo of whole meal wheat flour, and in order to lighten her a considerable portion of her load was removed and was subsequently consumed by the people in the adjacent districts. The result was that no case of beriberi was reported in that region for a period of one year following this event.

Parker [28] has reported an outbreak among the prisoners in a jail at Elizabeth, N. J., where white bread had been used as the main staple of the diet.

More recently cases of beriberi have been reported among the troops at Kut-el-Amara during the siege from December 4th, 1915, to April 29th, 1916. During a large part of the time the British garrison were restricted to white flour or biscuits and tinned meat or horseflesh, and many fell a victim to the disease. The Indian troops, on the other hand, ate either barley or " atta " flour (containing the aleurone layer of the wheat grain), which served to protect them against beriberi, although owing to their refusal to eat meat they suffered from scurvy from which the British were free.[29]

Two forms of beriberi are described, the " wet " beriberi in which there is much swelling of the tissues, and the " dry " beriberi in which all the muscles of the body are atrophied and the patient becomes entirely helpless. In both forms the earliest symptoms are loss of appetite, fatigue, depression, and slight fever. Vomiting and diarrhoea are frequently reported, and **Barbe** [30] found a severe duodenitis in a number of his fatal cases. **Riddell, Smith** and **Igaravidez,**[31] reporting on 60 cases of beriberi at an army base hospital at San Juan state, however, that gastric disturbances were uncommon, only seven cases of nausea being reported and these at the onset and for a few days only. In their experience the symptoms began in almost every case with a numbness over the legs, usually over the shins first and gradually spreading over the calves and upward to the abdomen.

[28] **Parker,** Pub. Health Repts. 29, 339, 1914.

[29] For further details of epidemics reported from 1891 to 1917 see **De Mello, Loundro** and **Rabello,** Anals. Scient. da Facul. de Med. do Porto, 4, 6, 1917.

[30] **Barbe,** Arch. Med. Pharm. Nav. June, 1918, quoted by **Jelliffe,** J. Nerv. Ment. Dis. 49, 522.

[31] **Riddell, Smith,** and **Igaravidez,** J. Am. Med. Ass. Feb. 22, 1919.

Pain was invariably observed in the calf muscles, these being usually the first to be affected. Only eleven patients showed any fever, and in these cases it was slight and lasted only a few days.

In the later stages there is great exhaustion, difficulty in breathing, multiple neuritis, paralytic symptoms, frequently followed by death.

The symptoms observed in fowls and pigeons are very similar. Within three weeks birds on an exclusive diet of polished rice usually show signs of weakness, followed very quickly by paralysis. Their necks are retracted, and they roll helplessly round their cages. Without treatment death follows very shortly, but with proper treatment their recovery is dramatic in its suddenness. Microscopic investigation of the tissues of a polyneuritic bird shows extensive peripheral degeneration of the nerve cells, even in the case of fowls which are killed before the symptoms of neuritis have manifested themselves.[32] **McCarrison**[33] finds that the oedema (swelling) characteristic of "wet" beriberi appears in the avian polyneuritis also, and is associated with pronounced increase in the size of the adrenal glands and greatly increased production of adrenalin, although the amount of adrenalin per gram of gland is actually slightly less in wet beriberi than in dry beriberi or in normal health. In 100 per cent of the cases of wet beriberi the quantity of adrenalin greatly exceeded that found in health, and in 83 per cent of these cases exceeded that found in dry beriberi.

Sicard, Roger and **Rimbaud**[34] point out that the cerebellar symptoms common in polyneuritis of fowls are not seen in human beriberi, and for that reason they are inclined to doubt whether the avian polyneuritis is actually identical with human beriberi.

The same general condition may be induced in swine,[35] rats,[36] dogs and cats.[37] According to **McCarrison,**[38] although a diet of autoclaved milled rice is fatal to monkeys, these animals die without developing the typical symptoms of polyneuritis, but **Gibson**[39] reports that of three monkeys fed on a mixture of white rice, with the addition

[32] **Vedder** and **Clark,** Phil. J. Sci. 7B, 423.

[33] **McCarrison,** Proc. Roy. Soc. 91b, 103, 1920.

[34] **Sicard, Roger** and **Rimbaud,** Par. Med. Dec. 1, 1917.

[35] **Hart** and **McCollum,** J. Biol. Chem. 19, 373, 1914; **Hart, Halpin,** and **McCollum,** *Ib.,* 29, 66, 1917; **Rommel** and **Vedder,** J. Ag. Res. 5, 489, 1915; J. Ind. Eng. Chem. 8, 199, 1916; **De Lacerda,** Berl. klin. Wechnschr. 23, 159.

[36] **Steenbock, Kent** and **Gross,** J. Biol. Chem. 35, 61, 1918; **Emmett** and **Allen,** Proc. Soc. Biol. Chem. J. Biol. Chem. 41, liii, 1920.

[37] **Karr,** J. Biol. Chem. 44, 277, 1920; **Voegtlin** and **Lake,** Am. J. Physiol. 47, 558, 1918.

[38] See p. 173.

[39] **Gibson,** Phil. J. Sci. 8B, 351, 1913-14.

of sufficient salt mixture to make up for the mineral deficiencies of the rice, one developed typical symptoms of beriberi, while the other two lost weight and suffered from malnutrition, but without neuritic symptoms.

Voegtlin and **Lake** [40] describe the following symptoms produced in cats and dogs by an exclusive diet of lean beef heated for three hours at 120° C. in the presence of alkali: Diminution of appetite, constipation, loss of body weight, weakness and sometimes drowsiness, followed by paralytic symptoms, tonic convulsions, spasticity of certain groups of muscles and disturbances of the circulation and respiration.

More recently, **Karr** [41] induced severe polyneuritis in dogs by feeding them vitamin-free diets of casein, sucrose, lard, bone ash and salt mixture. The symptoms induced were comparable with those observed by **Voegtlin** and **Lake**. The animals first vomited, became inactive and refused their food, then lost the power of walking or even standing. There were often tremors and twitching, with the head in motion, and occasionally severe tetanic convulsions.[42]

Weill and **Mouriquand** [43] report that in addition to the acute form of polyneuritis which develops in fowls after 20 to 25 days there is a chronic form which develops more slowly and which may be produced by a diet consisting of a mixture of raw and sterilized cereals (barley, corn and rice). The chronic condition was characterized by initial paralysis of the wing followed in some cases by paralysis of the feet. These symptoms were associated in certain instances with degeneration of the bones. The symptoms did not disappear when the sterilized cereal in the ration was replaced by raw cereal, nor did they yield to therapeutic administration of the portions of the cereals containing the antineuritic vitamin. In the acute experimental polyneuritis recovery was observed within a few hours after ingestion of raw cereal, bran or injection of vitamin. The investigators conclude that in the acute form the paralysis was due solely to a disturbance of the nerve centers which is chemical in its nature, whereas the chronic form appeared to be dependent on profound nerve degeneration. The paralysis was "functional" in the former case, and "lesional" in the latter. These observations are confirmed by **Lapicque** from his own experiments with mice. **Suguira's** observations [44] that extracts of fresh carrots and the crystalline preparations

[40] **Voegtlin** and **Lake**, Am. J. Physiol. 47, 558, 1918.

[41] **Karr**, J. Biol. Chem. 44, 277, 1921.

[42] For further details of the effect of neuritis-producing diets see p. 171 and ff.

[43] **Weill** and **Mouriquand**, C. r. soc. biol. 81, 432, 1918.

[44] **Suguira**, J. Biol. Chem. 36, 196, 1918.

from yeast may cure polyneuritis in those cases where the disease has developed quickly (in about 20 days), while these substances were powerless in cases where the symptoms came on slowly, might also be interpreted as indicating two distinct types of disease.[45]

As has been pointed out above, the earliest theory of the origin of beriberi was that of an infection. This theory passed into general disrepute as the relation between the disease and the diet was clearly demonstrated by the work of many investigators. Moreover, all efforts to transmit the disease from a polyneuritic animal to a healthy animal on normal diet or to trace the spread of infection from sick to healthy have failed.[46] Nevertheless there are still a few adherents of the infection theory. **Wydooghe**[47] describes an epidemic occurring in the African valley of Lukuga in which certain areas or centres of infection appeared to exist. Alteration in the diet in these centres did not cut short the epidemics, and the diet is said to have been practically uniform throughout the region at all seasons, in spite of which the disease was strictly localized and periodic. Also, outbreaks occurred apparently as the result of camping on old grounds which had previously been marked beriberi centres. **Wydooghe** believes the disease is due to an infecting germ which is possibly carried by some insect agent, and which is present in the blood for a short period only, so that the propagation of the disease is difficult outside of the epidemic centres and where the carrier is absent. **Barbe**[48] likewise finds a purely dietetic hypothesis unsatisfactory, and is inclined to attribute the disease to a microbic infection, possibly involving the duodenal passages, which is favored by an intoxication derived from the food and by unhygienic surroundings. **Sprawson**[49] believes that the conditions known as beriberi may arise from different causes under different circumstances, the clinical appearance being approximately the same in all cases. One class of cases is due to a vitamin deficiency, while another appears to result from an infection.[50]

McCarrison[51] endeavored with some apparent success, to isolate an organism which would induce beriberi, but **Williams** and **Johnston**[52] failed to confirm his results. He himself concluded that vita-

[45] See also **Williams**, J. Biol. Chem. 25, 437, 1916.

[46] **Breinl**, J. Trop. Med. 19, 129, 1916; **Sicard, Roger,** and **Rimbaud**, Paris, Med. Dec. 1, 1917.

[47] **Wydooghe**, Bull. Soc. Path. Exct. March, 1918.

[48] **Barbe**, Arch. Med. et Pharm. Nav. June, 1918.

[49] **Sprawson**, Quart. J. Med. 13, 337, 1920.

[50] See also **Hopkins**, Roy. Soc. Med. Sect. Ther. Pharm. Lanc. 1919, ii, 979.

[51] **McCarrison**, Ind. J. Med. Res. 2, 369, 1914.

[52] **Williams** and **Johnston**, Phil. J. Sci. 10B, 337, 1915.

min deficiency was an essential factor in the origin of beriberi, but that such deficiency is so rarely complete as to be the sole agent responsible for it, and infectious and parasitic agents are often important causes determining the onset of the symptoms. Bacterial organisms, of whatever kind, that may be isolated from the blood in human beriberi, may invade the blood and tissues under conditions of dietetic deficiency, and thus convert a state of potential into one of active disease. Since such organisms are not the true cause of the malady they cannot be expected to produce it in inoculation experiments. "They are but weeds which flourish in the soil made ready for them by dietetic deficiency." [52a]

Shibayama [53] came to somewhat the same point of view from his study of beriberi in Japan. While a monotonous and one-sided diet may give rise to the onset of the symptoms, he is unwilling to grant that this is the true cause of the disease. The symptoms and anatomical changes seem to him to develop from intoxication by a poison which is produced by a certain micro-organism in the body, especially in the intestine.

Lebredo [54] has reported experiments which demonstrate the existence of an extremely persistent bacterial organism which he believes may be responsible for the production of a "beriberi toxin," but these experiments also lack confirmation at present.

The early investigators who studied the connection between beriberi and a polished rice diet, noting the resemblance between the polyneuritic symptoms and those of an intoxication, reached the conclusion that polished rice contained some toxin either inherent in the rice or produced by the action of moisture or a fungus growth.[55] It has been frequently demonstrated, however, that the onset of the disease is not dependent in any way upon the use of spoiled or fermented rice, and the theory of a toxic substance naturally present in the rice grain was entirely disproved by the work of **McCollum** and **Davis**,[56] who showed that on the one hand substitution of dextrin or

[52a] **Clair** (Bull. soc. pathol. expt. 13, 191, 1920) claims the conditions produced in pigeons by feeding with polished rice have no connection with beriberi. In human beings the disease is alleged to be due to toxic products of bacterial decomposition of rice. It is thus regarded as an infectious disease, though the agent has not yet been isolated.

[53] **Shibayama,** J. Trop. Med. Hyg. 16, 283, 1914.

[54] **Lebredo,** Proc. 2nd, Pan. Am. Sci. Cong. Sect. 8, pt. 2, p. 29.

[55] **Braddon,** "The Cause and Prevention of Beriberi," 1907; **Couzien,** Ann. hyg. med. colon. 15, 445; Zentr. Bioch. Biophys. 14, 909; **Caspari** and **Moskowski,** Berl. klin. Wchnschr. 1913, 1, ii, 1515; **Williams** and **Johnston,** Phil. J. Sci. 10B, 337. 1915; **Goto** and **Takahata Chuo,** Igaku-Zasshi, 25, 575, 1918, quoted by **Izume,** J. Tok. Chem. Soc. 41, 225, 1920.

[56] **McCollum** and **Davis,** J. Biol. Chem. 23, 181, 1915.

purified casein for half the ration of polished rice caused no improvement in the condition of rats who had begun to decline on a diet of rice alone, and, on the other hand, no toxic effect was produced by the inclusion of as much as 80 to 90 per cent of polished rice in the diet, provided egg, milk powder, or wheat embryo were fed at the same time.

A variation of this theory of intoxication is found in the hypothesis of **Walshe**,[57] who believes that there are two factors responsible for beriberi, one of which is vitamin deficiency and the other an intoxication due to the abnormal hydrolysis of carbohydrates with the formation of toxic products. According to **Walshe** the beneficial effect of the rice polishings and similar curative substances is due to the vitamins present which play some undetermined rôle in regulating the carbohydrate hydrolysis, while the earlier intoxication theories assume the presence of some substance in the bran which neutralized the toxins of the rice.

Schaumann [58] noted an abnormality in the phosphoric acid content of the urine of beriberi patients, and concluded that beriberi was connected with faulty phosphorus metabolism, a view with which **Moskowski** [59] is in accord.[59a] The evidence on which this view is based is, however, capable of another interpretation.[60]

In 1911, **Funk** took up the study of the disease and developed his vitamin theory according to which beriberi and polyneuritis are caused by deficiency of vitamin *B*.[61]

[57] **Walshe,** Quart. J. Med. 11, 320, 1918.

[58] **Schauman,** Arch. Schiffs-u-Tropenhyg. 12, 683; Chem. Zentr. 1908, ii, 1056.

[59] **Moskowski,** Arch. Schiffs-u. Tropenhyg. 15, 653, 1912-13.

[59a] The belief that in the absence of vitamin *B* and the subsequent development of beriberi there is a breakdown in metabolism, more particularly in regard to the metabolism of carbohydrates, has led **Findlay** (Biochem. J. **15**, 104, 1921) to determine the glyoxylase content of the liver in avian beriberi; for glyoxylase plays an important part in carbohydrate metabolism. The results obtained suggest that in pigeons suffering from beriberi there is, as compared to control birds, a reduction in the glyoxalase content of the liver. The administration of vitamin *B* produces a definite increase in the amount of glyoxylase.

[60] See p. 79 on the relation between vitamin and P_2O_5 contents in grains.

[61] **Funk** and **Cooper,** Lanc. 1911, ii, 1266; **Funk,** Trans. Soc. Trop. Med. 5, 86, 1911; J. Physiol. 43, 395. 1911; *Ib.,* 44, 50; *Ib.,* 45, 75, 489, 1912; *Ib.,* 46, 173; Proc. Physiol. Soc. J. Physiol. 47, xxv., J. Physiol. 48, 228, 1914; *Ib.,* 53, 247, 1919-20; **Funk** and **von Schonborn,** *Ib.,* 48, 328, 1914; **Funk** and **Douglas,** J. Physiol. 47, 475; **Funk,** Ergebniss. d. Physiol. 13, 125, 1912; Z. physiol. Chem. 88, 352, 1912; 89, 373, 378, 1913; J. State Med. 20, 341, 1913; Parm. J. 89, 351; Brit. Med. J. 1913, i. 814; Bioch. J. 7, 81, 211, 1913; **Funk** and **Macallum,** *Ib.,* 7,

Rice as it comes from the thresher ("paddy" rice) is enclosed in a hull which must be removed by grinding the rice between stones, or, as in Burmah, in a larger mortar. The grain produced by this opera-

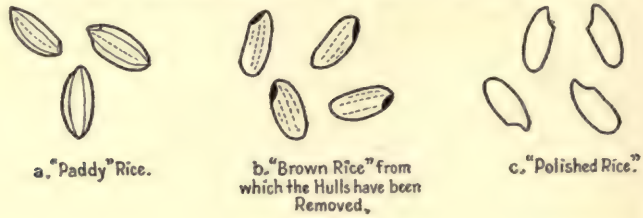

FIG. 8

tion is brownish in color and consists of two parts, the endosperm and the embryo or germ. The whole is surrounded by an outer layer, the pericarp or "silver-skin," next to that is a layer rich in protein, known as the aleurone layer, and within this lie the starch grains. In a little pocket formed by the aleurone layer at the end and to one side is the embryo. In order to remove the brown coat and thus meet the demand for a whiter rice grain millers have had recourse to more or less elaborate machines in which the grain passes through leather rollers which rub off the outer layer, the embryo becoming separated from the endosperm at the same time and being discarded with the polishings.

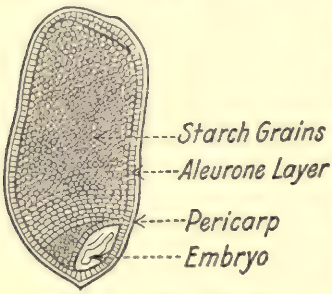

FIG. 9.— Rice Grain

Polished rice has the advantage that it keeps better than unpolished rice, which is likely to be infested with weevils and other insects.[62]

The change in the chemical composition of the rice due to the polishing process is shown in the following table:[63]

	Hulled Per cent	Polished Per cent
Water	12.00	12.40
Protein	7.20	6.90
Fat	2.00	0.40
Starch, sugar, and gum	76.80	77.40
Cellulose	1.00	0.40
Ash	1.00	0.50

356, 1913; Z. f. physiol. Chem. 92, 13, 1914; Naturwiss. 2, 121, 1914; Die Vitamine, Weisbaden, 1914; **Funk** and **Macallum**, J. Biol. Chem. 23, 413, 1915; *Ib.*, 27, 1, 1916; **Funk, Lyle,** and **McCaskey,** J. Biol. Chem. 27, 173, 1916; **Funk,** Bioch. Bull. 4, 304, 1915; 5, 1, 1916; **Funk** and **Dubin,** Sci. 52, 448, 1920.

[62] See **Ottow,** "Testing, Storage, and Preparation of Unpolished Rice," Natuur. Tyjds. Ned. Indie, 74, 143, 1915.

[63] U. S. Dept. Agriculture, Bureau Chem. Bull. 45.

Protein, fat, and mineral matter are all reduced by this process, and in addition the vitamin *B* which is present in the germ and in the pericarp and aleurone layer of all cereals [64] is entirely removed. According to **Funk** it is the loss of this factor which leaves polished rice so unsuited to form the chief part of the diet.[64a]

Vedder [65] suggests the existence of two vitamins, deficiency of one resulting in dry beriberi, and deficiency of the other causing wet beriberi. He bases this hypothesis on the fact that while the administration of unhydrolyzed extract of rice polishing to cases of wet beriberi results in the prompt relief of the symptoms, in cases of dry beriberi administration of this extract is followed by little or no improvement in the paralytic symptoms. The administration of **Funk's** base [66] to cases of dry beriberi produces an immediate improvement in these cases as well as in polyneuritis gallinarum, from which **Vedder** concludes that dry beriberi is caused by the deficiency of this substance in the diet, and that dry beriberi of man and polyneuritis gallinarum are essentially the same disease. Since wet beriberi, on the other hand, was cured by the extract represented by the filtrate from the phosphotungstic precipitate which does not contain **Funk's** base, **Vedder** concludes that wet beriberi is caused by deficiency of a different substance in the diet and that dry beriberi and wet beriberi are different diseases, deficiency of a separate vitamin being responsible for each.

McCollum and **Davis** [67] made a complete study of the nutritive deficiencies of polished rice and found that it must be supplemented with additional protein, mineral salts, and both vitamins *A* and *B* in order to secure normal development. Experiments with other foodstuffs have shown that deficiency in any one of these factors is suf-

[64] See p. 78.

[64a] **Sawamura** (Japanese Patent 36,720, July 6, 1920) found the embryo of rice to afford the following analytical results: water 5.73, crude protein 24.30, crude fat 20.12, lecithin 0.93, sugars 10.79, starch 14.89, crude fiber 9.77, and ash 13.47 per cent. It contained a large quantity of vitamin. By treating with 0.2 per cent caustic soda solution the protein and fat, etc., in the embryo go into the solution; while, using 0.1 per cent hydrochloric acid, calcium phosphate, vitamin, etc., are obtained in the solution. The two solutions are mixed together and a small quantity of lime water is added. The precipitate thus obtained is collected, washed with water and dried. It contains a large quantity of vitamin and water 4.87, crude protein 41.55, crude fat containing lecithin 46.50, and ash 3.86 per cent, P_2O_5 1.19 per cent and CaO 1.00 per cent.

[65] **Vedder**, "Beriberi," N. Y. 1913; Proc. 2nd Pan Am. Sci. Cong. Sect. 8, Pt. 2, p. 26; **Vedder** and **Clark**, Phil. J. Sci. 7, 1912; **Vedder** and **Williams**, *Ib.*, 8B, 175, 1913.

[66] See p. 121.

[67] **McCollum and Davis**, J. Biol. Chem. 23, 181, 1915.

ficient to limit growth, but only in the absence of *B* are polyneuritic symptoms seen. A supply of this factor, either from rice polishings, wheat embryo, yeast, or some other source, brings about rapid recovery from the disease and provides protection against its onset or recurrence.

In the milling of wheat the bran and embryo of the wheat grain are removed as are those of the rice, although the process is different. The finer and whiter the grade of flour the more completely have the vitamin-bearing parts of the grain been removed. Where bread made from such flour forms a comparatively small part of a generous mixed diet this deficiency is unimportant, but where the bread forms the greater part of the diet, as was true of the cases in Labrador and Newfoundland reported by **Little** (l. c.) serious results may ensue.

The work of **Funk** and others on the isolation of the antineuritic substance from rice polishings and other sources has been referred to elsewhere.[68] While of great interest from the purely scientific point of view these contributious are much less important practically than the determination of the distribution of this substance in the natural foodstuffs.[69] Since it is found in abundance in the cheapest foods, cereals and legumes, provided only that these have not been robbed of their store by the misguided efforts of civilized man to " improve " them, there is no reason why even the poorest races of mankind should continue to suffer from the ravages of beriberi. Repeatedly change of diet has been shown to result in the eradication of the disease from a community.[70]

Vedder[71] points out that in the reports of cases which are supposed to discredit the dietary hypothesis the statements regarding the diet on which the disease has been developed are too vague to be of any value.

[68] See p. 121 and ff.

[69] See pp. 66–88.

[70] **Van Leent,** Arch. de Med. Nav. 1867, Oct. p. 241. Communication sur le Beriberi, Cong. Internat. d. Sc. Med. Amst. 1880, vi, 170. etc.; **Vorderman,** Onderzoek naar het verband tusschen den aard der rystvoeding in de gevangenissen op Java en Madoera en het voorkomen van beriberi onder de geinterneeden. Batavia, 1897; **Takaki,** Three Lectures on the Preservation of Health Amongst the Personnel of the Japanese Navy and Army. Lancet, 1906, 1, 1369, 1451, 1520, etc.; **Fletcher,** Journ. Trop. Med. and Hyg. 1909, xii. Lancet, 1907, i, 1776, etc.; **Highet,** Philippine Journ. Science 1910, v. 73; **Weiser.** Philippine Jour. Science 1911, vi, 1237. Also Jorn. Am. Med. Ass. 1911, LVI, 1238; **Theze,** Ann. d'Hygiene de Med. Col. 1910, xiii, 16; **Chamberlain,** Philippine Journ. Science, 1911, vi, 133; **Vedder,** Beriberi, William Wood and Co., New York, 1913.

[71] **Vedder,** Am. J. Trop. Dis. and Prev. Med. 1914, i, 826; Proc. 2nd Pan Am. Sci. Cong. Sect. 8, Pt. 2, p. 23.

"Any such communication to be worthy of attention should contain a detailed statement of the food actually consumed by the affected persons, for the 90 days prior to the development of the disease, showing components, quantities, and a statement as to whether the articles used were fresh, canned, or otherwise preserved."

While the antineuritic vitamin is so widely distributed that it is easy under ordinary circumstances to secure a diet which contains sufficient of it, there are times, — as in the rationing of armies in the field — when it is highly advantageous to have a concentrated supply. Where possible the use of whole meal flour from rye, wheat, or barley, or the liberal consumption of peas, beans, or lentils is recommended. **Hulshoff**[72] reports from his study of the dietetic treatment of beriberi in the Dutch West Indies, that large amounts of puree of peas had an unmistakably curative action on beriberi. As much as 150 g. of the puree three times a day was required, however, smaller amounts showing no benefit. Oatmeal had a similar influence, but 125 g. three times a day was necessary. Europeans required a little less. A native bean seems also to have a like action. It is to be remembered that these substances lose their efficacy with age, three-year-old beans having been found quite inert.

Nuts in general are rich in antineuritic, and **Grieg**[73] recommends ground-nut (peanut) meal biscuit as emergency rations for the troops.

Commercial wheat germ is highly efficacious, being three or four times as potent as dried peas or egg yolk which come next in order. A preparation of yeast produced under special conditions according to researches carried out under the British War Office and known as "marmite" was used as an antineuritic by the British troops in Mesopotamia during the war and was found to be equivalent to wheat germ in efficacy.[73a] Autolyzed yeast is more effective than pressed or dried

[72] **Pol,** Norsk. Mag. Laegevidenskaben 77, No. 1, 1916, J. Am. Med. Assoc. 66, 698.

[73] **Grieg,** Ind. J. Med. Res. 6, 143, 1918.

[73a] **Abderhalden** (Arch. ges. Physiol. 178, 260, 1920) induced polyneuritis in pigeons by continuous feeding upon polished rice, and the therapeutic effects of various fractions of yeast were measured. What Abderhalden terms the "nutramine" of dried yeast can not be completely extracted by absolute alcohol, by acetone, or by a combination of these two extractives. The extracts of yeast given to pigeons on a rice diet ameliorate certain symptoms of the disturbance but they fail to provide a complete regimen. Apparently there is some substance, of an insoluble nature, in the yeast which is effective in combating the disturbances associated with a polished rice diet.

Pigeons were fed for two weeks on a diet consisting exclusively of polished rice and at the end of this period they presented the characteristic manifesta-

yeast.[74] **Saleeby** [75] prepared an extract of autolyzed yeast by placing brewers' yeast, separated from the adhering beer, in an incubator at 35° C. for about 48 hours, filtering, and concentrating the filtrate under reduced pressure at a temperature below 60° C. to about one-third of the original volume. About 40 cases of beriberi (five of them children under two years) were treated with this extract, adults being given 15 to 40 cc. three times a day, children two to four cc. every three hours. All acute symptoms of neuritis were affected in less than three days. Infantile symptoms were cured with comparative rapidity. Hydrolyzed extract of rice polishings produce a similar and even more pronounced effect.[76]

---

tions of alimentary dystrophy. During the period of the experiment, body weight, temperature and gas metabolism determinations were made and curves which were plotted for the values secured show a gradual fall throughout the course of the experiment. When yeast, or an alcoholic extract of yeast, was added to the diet, a rise in the gas metabolism always occurred. (Arch. ges. Physiol. 1921, 80.)

[74] **Seidell,** J. Biol. Chem. 29, 145, 1918.

[75] **Saleeby,** Phil. J. Sci. 14, 11, 1919.

[76] For preparation of concentrated vitamin extracts see Ch. VII.

Simonnet (Bull. soc. hyg. 9, 69, 1921) presents data obtained in feeding experiments with pigeons. The "complete diet" used consisted of the following: Meat residue 11 g., peanut oil 5 g., potato starch 59 g., salt mixture 4 g., filter paper 5 g., agar 5 g., butter 10 g., brewers' yeast 1 g. The butter furnished *A*, whereas the yeast was the only source of *B* and *C*. This diet permitted satisfactory maintenance of the birds during the experimental period of 170 days. The same ration but without yeast did not permit maintenance of the birds. Characteristic symptoms of polyneuritis appeared.

The respiratory quotient was studied by **Jansen** and **Mangkoewinoto** (Med. geneesk. lab. Weltevreden [Java] 1920, 3e Ser. A, Nos. 1, 2 and 3, 50; Physiol. Abs. 5, 361; Chem. Abs. 1921, 2665) with the hope that the fall in respiratory quotient might be made use of in testing foods for antineuritic vitamin. The respiratory quotient did fall in one or two cases, but the fall is not constant and reliance cannot be placed on it in the diagnosis of polyneuritis.

When fowls and cats were fed on polished rice and food deficient in vitamins, the organs did not show any change in sensitivity towards adrenaline, histamine, choline, and atropine. (**Van Leeuwen** and **Verzar,** Chem. Abs. 1921, 2658).

Fitch (Am. Med. 16, 369, 1921) reports a series of experiments in which beriberi was produced in pigeons by feeding polished rice.

CHAPTER XII

SCURVY

Scurvy has long been the dread of navigators, explorers, and armies in the field; in fact wherever men are cut off from available supplies of fresh food its ravages are known. Babies fed exclusively on sterilized foods suffer from the form known as "Barlow's disease," and as **Holst** first pointed out symptoms identical with those of human scurvy can be produced in guinea pigs by feeding on a restricted diet.[1]

The first distinct account of the disease is contained in the history of the crusade of Louis IX in the Thirteenth Century, during which the French army suffered greatly from it. In the Sixteenth Century it was endemic in various parts of northern Europe. During **Cartier's** second voyage to Newfoundland in 1535 one hundred out of his crew of 103 men developed symptoms of scurvy, but were cured by the administration of a decoction of fresh pine needles. In 1593 Admiral **Hawkins** stated that within his personal experience not less than 10,000 seamen had died of scurvy, and an appalling case is on record of the Spanish ship "Oriflamma" which was found adrift with her entire crew dead of scurvy.

In adults the symptoms come on gradually, the onset being marked only by a certain failure of strength which is manifested when any effort is called for. Breathlessness and exhaustion are easily induced, along with a corresponding mental depression. The face is sallow and pallid, the eyes sunken, and the gums tender. The muscles of the body and limbs become painful with vague pains suggesting rheumatism. Appetite and digestion may be unimpaired in the earlier stages, but gradually decline. As the disease progresses the gums become livid, spongy, ulcerating, and bleeding, the teeth are loosened and drop out, the breath becomes excessively foul. Effusions of blood from the vessels in the skin and other tissues takes place either spontaneously or as the result of slight pressure or injury to the skin. These are frequently so extensive as to cause swelling of the muscles in which they occur, giving the appearance of extensive bruises and

[1] **Holst and Frölich, J. Hyg. 7, 634, 1907.**

tending to become hard and brawny. In addition there is bleeding from the mucous membranes of the nose, eyes, and alimentary and respiratory tracts, and hemorrhages into the pleural, pericardial or peritoneal cavities. According to **Hess**,[2] the coagulability of the blood is almost normal, the escape of blood from the vessels being due to a weakening of the walls or to a lesion of the endothelial cells. Ulcers are apt to break out in the limbs. In the later stages exhaustion becomes profound, with a tendency to syncope, and various complications such as diarrhoea and pulmonary or kidney troubles are liable to appear, any or all of which may bring about a fatal result. Diminished secretion of urine is a common symptom of both beriberi and scurvy.

The protein metabolism is disturbed during the progress of the disease as is evidenced by the increased ammonia and amino acid output.[3]

Stefanson[4] gives the following description of cases observed by him during 1916-17:

"**Anderson** complained to me of having been gradually becoming more and more unwell for a week or two. The first symptoms noted by him was dizziness on suddenly standing up, 'laziness,' gloom and irritability, showing itself in a tendency to condemnatory and uncalled-for argumentativeness, proneness to becoming tired, and loosening of the teeth and a swelling and recession of the gums, with a dull local ache in the gums or roots of the teeth. The appetite was normal both as to quality and kind of food desired. . . . At this time the teeth of the men were so loose that they could be plucked out with the fingers with no effort, and the gums were of such a cheese-like consistency, that they were cut (with little bleeding) by wooden toothpicks about as easily as an ordinary 'American' cheese could be. Every joint was sore and all movements painful."

Hess[5] points out that the association of circulatory disturbances with latent scurvy, which has been overlooked until lately, furnishes a clinical link between scurvy and beriberi. One of the earliest symptoms is frequently tachycardia, a heart beat of 140 or 150 being quite common in infants. Still more noticeable and characteristic is the instability of the pulse rate, which may increase by from 20 to 40 beats to the minute on the slightest exertion or rise in temperature. Accompanying this may be respirations rising to 40, 50, or even 60 to the minute. These symptoms, to which **Hess** gives the name " cardio-

[2] **Hess,** J. Am. Med. Ass. 76, 693, 1921.

[3] **Labbe, Haguenea** and **Nepveux,** Bull. mem. soc. med. Hop. 44, 1094, 1920, Chem. Abs. 1920, 3267; See also **Brandt,** Arch. f. Kinderheil. 67, 395, 1920. The blood in scurvy.

[4] **Stefanson,** J. Am. Med. Ass. 71, 1715, 1918. [5] **Hess,** *l. c.*

respiratory phenomena," yield promptly to antiscorbutic treatment, from which **Hess** infers at least a functional relation between the antiscorbutic vitamin and nerve tissue. The scorbutic organism, even in the latent stages of the disease, is peculiarly sensitive to infection. **Abels**[6] found that scorbutic guinea pigs succumbed to much smaller peritoneal injections of cultures of the slightly toxic *B* coli than did non-scorbutic animals. Intracutaneous injections of staphylococci, toward which guinea pigs are normally very resistive, caused distinctly palpable infiltrates, and in one case an abscess, in scorbutic pigs, while the controls presented at the most a very slight infiltrate. He also found support for the assumption that the hemorrhages observed in scurvy are brought about by the toxic effects of infections.

Barlow's disease was first observed in England in 1876 by Sir T. **Smith** and later fully investigated by Sir **Thomas Barlow**. It is seen principally in children from 6 to 18 months. The child is sallow and fretful before any marked physical signs appear.[7] Later there appears a sensitiveness to touch, particularly about the lower limbs, and the child will refuse to move or bear any weight on the legs. Later the limbs swell, the tenderness increasing. The growth in weight and length is markedly affected.[8] **Comby**[9] urges the importance of suspecting scurvy in an infant whenever it is observed to keep its limbs still and cry if they are touched. This is said to be a constant symptom in nurslings, but not in older children. The progress of the disease is marked by great and progressive anemia, apathy, spongy gums, hemorrhages into various structures, particularly under the periosteum and muscles, with suggestive thickenings around the shafts of the long bones, producing a state of pseudo-paralysis.[9a]

While the acute stages of infantile scurvy are comparatively rare, incipient cases are more common, and not always easily recognized. **Comby**[10] reports that 90 per cent of the 72 cases of this disease which

[6] **Abels,** Wien. klin. Woch. 33, 899, 1920.

[7] See **Hess and Fish,** Am. J. Dis. Child. 8, 386, 1914.

[8] **Hess,** Am. J. Dis. Child. 12, 152, 1916.

[9] **Comby,** J. Méd., Paris, 1920, 1, 673.

[9a] Seven children were cured of Barlow's disease by feeding them exclusively upon milk. In five of the cases the milk was heated for 10 to 35 minutes and in two cases the heating was prolonged to one hour. It is concluded that the vitamin is not as readily affected by heat as has been assumed. The concentration of the food, i. e., the water content, is important. **Nobel** (Z. Kinderheilk. 28, 348, 1921).

Morikawa (Igakkwai 19, 9, 1920; Endocrinology 4, 615) concludes that the hypertrophy which is similar to that found in polyneuritis gallinarum indicates that the absence of a necessary vitamin is also an etiological factor in Barlow's disease.

[10] **Comby,** Bull. Soc. Med. Hop. Par. 45, 288, 1921.

he encountered had been wrongly diagnosed by the attending physician. **Tobler**[11] states that in addition to the infants suffering from manifest scurvy there are a far larger number of latent cases with characteristic muddy complexion, lack of appetite, stationary weight, and fretful disposition.

In a discussion of this disease **Hess** remarks:[12]

Infantile scurvy differs clinically from the other deficiency diseases mainly in the fact that it is characterized by the production of hemorrhage in various parts of the body, hemorrhage into the gums, into the skin, beneath the periosteum, into the kidneys, etc. A study of the pathogenesis of these hemorrhages has shown that they were not the result of alterations of the blood itself, a delayed coagulability, or a decrease of blood platelets, but that they were due to the alteration of the blood vessels which allow the blood to traverse its walls. This alteration probably should be regarded merely as a part of the general tissue changes which occur in this disorder.

Although hemorrhage is not encountered in beriberi, a careful consideration will show some clinical relationships between these two diseases. As is well known, signs of involvement of the nervous system are the characteristic manifestations in beriberi. A study of the cases which came under our view showed that infantile scurvy is not entirely free from nervous signs. The knee jerks are increased, there seem to be slight involvement of the optic disks in some cases and perhaps sensitiveness of the cutaneous nerves. Again, dilation of the right heart, a remarkable pathological condition which has been frequently described in beriberi and noted by **Andrews** in infants who had suffered from this disease, was found to occur likewise in infantile scurvy, as demonstrated in numerous Roentgen ray examinations. Oedema, which is such a common symptom in beriberi, is not frequently present in infantile scurvy. In fact, there is one form of this disorder, an exceptional type, where oedema is most marked. In this connection it should be remembered that degeneration of the nerves has been found by **Ingier** in some animals suffering from scurvy, that **Holst** and **Frölich** have noted that guinea pigs fed on decorticated or highly milled rice, developed scurvy and not beriberi, and that **Darling** has reported that in some African negroes a diet that caused scurvy in one set of men, caused neuritis in others. There is evidently, therefore, a definite interweaving in the symptomatology of these two deficiency diseases. As is well known, beriberi is produced by a diet of decorticated rice and may be cured by feeding the rice polishings. In this regard, there seems also to be some connection between the two diseases, for we found that the giving of wheat middlings, which may be regarded as the pericarp of the wheat, in some instances resulted in a prompt amelioration of the symptoms, although it was unable to bring about a complete cure of the disorder.

Certain of the lower animals, notably guinea pigs, are extremely susceptible to scurvy, while other species such as the rat and the prairie dog appear to be immune.[13] Whether this immunity is real

[11] **Tobler**, Ztschr. f. Kinderh. 18, 63, 1918.

[12] **Hess**, Proc. 2nd Pan. Am. Sci. Cong. Sec. 8, Pt. 2, p. 49.

[13] **McCollum and Parsons**, J. Biol. Chem. 44, 603, 1920; **McClendon, Cole,**

or only apparent is an interesting question, but one which is difficult to settle, since the only test known for the presence of the antiscorbutic factor in foods is the biological method and this may not be sufficiently delicate to detect small quantities present. A very interesting investigation has recently been made by **Parsons** [14] who has been able to show that the livers of rats fed on a typical antiscorbutic ration for from 213 to 247 days were high in antiscorbutic, as shown by the guinea pig test. Scarcely any difference in antiscorbutic content was demonstrable between the livers of these rats and those of control animals on a normal diet. This seems to show that the rat actually requires the factor for its metabolism, but whether it is synthesized in the body of the animals or received in small amounts in the food is at present unanswerable. Preliminary experiments are said to suggest the existence of the same phenomenon in newly hatched chickens. **Plimmer** [15] notes that pigs fed on cooked food, consisting of a mash composed of meals, sharps or middlings and turnips, developed scurvy. The animals were cured by giving them the same food but in the raw condition and by increasing the quantity of turnips, which are rich in the antiscorbutic factor.

The symptoms of guinea pig scurvy have been very carefully noted by various observers. A full description is given by **Cohen** and **Mendel** [16] who remark as follows:

> In the guinea pig this is a disease the onset of which is usually characterized first by a tenderness of the joints; and the wrists, ankles and knees become involved in the order named, though there are many exceptions. There follows in a day or so, a gradual swelling of the affected joints, often to twice or three times the original diameter of the bone. Sometimes in the younger animals a joint will fracture spontaneously, — the wrist being most susceptible in this regard. The older animals develop a difficulty in using their hind legs, which seem to become stiffened or paralyzed. In order to relieve the pain in the affected member, the animals will lie on their side or back and assume the scurvy position described by **Chick** and **Hume**.[17] If curative measures are instituted early enough, the swelling disappears entirely; if not, these knobs harden into exostoses that can be felt through the skin. The disease appears also to affect the junctions of the ribs with the cartilages, and

---

Engstrand, and **Middlekauff**, *Ib.*, 40, 257. 1919; **Funk**, *Ib.*, 25, 409, 1916; **Harden** and **Zilva**, J. Path. Bact. 1919; **Holst** and **Frölich**, Zeitsch. f. Hyg. Infek. 72, 1, 1912; J. Hyg. 7, 634, 1907; **Drummond**, Bioch. J. 13, 77, 1919.

[14] **Parsons**, J. Biol. Chem. 44, 587, 1920.

[15] **Plimmer**, Biochem. J. 14, 570, 1920.

[16] **Cohen** and **Mendel**, J. Biol. Chem. 35, 426, 1918. See also **Givens** and **Cohen**, *Ib.* 36, 132, 1918.

[17] Tr. Soc. Trop. Med. Hyg. 10, 141, 1917.

in advanced cases it is possible to palpate these costochondral enlargements described by **Jackson** and **Moore**.[18]

Accompanying the joint enlargement, one notices a marked hypersensitiveness that is succeeded by a dullness or lethargy. The symptoms described may appear while the animals eat well and gain in weight steadily. Then follows a loss in appetite with a resulting decline in weight to death.

Hemorrhage of the gums in scorbutic guinea pigs has been reported by **McCollum** and **Pitz**, and **Jackson** and **Moore** have found it in a few of their animals. However, **Givens** and **Cohen**, in common with **Holst** and **Frölich**, and with **Gerstenberger**, never observed this feature. Very rarely (in four cases) there was an appearance of submucous hemorrhage at the base of the lower incisors. Oftener (in ten or fifteen cases), there was observed a hyperemia or congestion at the same site.

Loosening of the teeth was quite a common occurrence in the animals with scurvy. Occasionally during life, the lower incisors were shown to be loose. Sometimes they could break off, though never in normal pigs or in animals with scurvy that was recently developed. At autopsy the lower molars were nearly always loose,— at times to such a degree as to permit the teeth to be removed easily with forceps. The upper molars on the contrary were not so often affected in this manner.

The X-ray picture of the affected joints is reported to be characteristic of the disease. As first shown by **Fraenkel**, there appears at the junction of the epiphysis and diaphysis of the long bones a white line which recedes very slowly after other symptoms have gone.

At autopsy the two most noticeable conditions are hemorrhage and fragility of the bones. The viscera often were normal in appearance; though now and then the stomach, intestines, or cecum showed congestion, hemorrhage or ulceration. These would occur usually on the oat and water or oat and milk diet, and less frequently on the soy bean diet.

The similarity between the symptoms thus described and those of human scurvy leave no room for doubt as to the identity of the disease in both species.

Hess and **Unger** [19] point out that all work on this subject should be checked by careful histological examination of the bones. Changes occur in the epiphyseal ends of the bones which are typical of scurvy and differ from those which occur in other diseases of the bony system. It is, therefore, imperative that such lesions be found before we take it for granted that we are really dealing with scurvy. Hess and Unger have encountered microscopic lesions at autopsy which were considered characteristic of true guinea pig scurvy (hemorrhages in to the costochondral junctions of the ribs and swelling of the joints) but which later microscopic examination proved to resemble rickets. The bones of the animals referred to in this study showed the typical "frame work" marrow at the epiphyseal junction, a marrow poor in cells and rich in connective tissue; it did not show the osteoid tissue always associated with rickets.

[18] **Jackson** and **Moore**, J. Infec. Dis. 19, 478, 1916; J. Am. Med. Ass. 67, 1931, 1916.

[19] **Hess** and **Unger**, J. Biol. Chem. 35, 473; 35, 479, 1918.

Zilva and **Wells** [20] have described the changes observed in the structure of the teeth of scorbutic guinea pigs. Even where no microscopic signs of the disease had been detected during the life-time and only the faintest traces could be discovered with the naked eye at post-mortem, well-defined microscopic changes were invariably detected in the structure of the teeth. In advanced cases of scurvy the teeth were found to be apparently sound but useless, inasmuch as they had been loosened by the gradual absorption of the cement membrane of the alveolar sockets, leaving the portion below the neck exposed. Probably pereostitic pain accompanies this condition as is the case with human patients. In addition, the teeth presented all the appearance of senile change.[21]

The condition in young guinea pigs was precisely the same as in the older animals, and the same changes have been observed in monkeys.

The circumstances under which scurvy makes its appearance points as indicated, to a dietetic origin for this disease, a hypothesis which is further supported by the beneficial effects of fresh foods or certain preserved fruit juices. Nevertheless certain other theories have been advanced from time to time.

Wright [22] claimed that the symptoms of scurvy were caused by acidosis, a theory which has never found much acceptance [23] and which is entirely disproved, so far at least as guinea pig scurvy is concerned, by the study of the alkaline reserve of the blood in scurvy made by **McClendon, Cole, Engstrand,** and **Middlekauf.**[24]

Chronic poisoning by tainted meat and fish was held responsible by **Jackson** and **Harley** [25] a theory which found some acceptance when first promulgated, but the experimental evidence on which it was based has not been supported by later work and is itself susceptible of other interpretation.[26]

[20] **Zilva** and **Wells,** Proc. Roy. Soc. 90B, 505, 1919.

[21] **Talbot** (Dental Cosmos, 63, 795, 1921) considers interstitial gingivitis (pyorrhea alveolaris), its occurrence as a deficiency disease, and its relationship to scurvy.

[22] **Wright,** Army Med. Dep. Rep. 37, 394, 1895.

[23] See **Furst,** Norsk. Mag. Lag. 1912, 1; **Holst** and **Frölich,** Z. Hyg. 72, 1, 1912; **Funk,** J. Biol. Chem. 25, 409, 1916; **Labbe, Haguenea,** and **Nepveux,** Bull. mem. soc. me. Hop. 44, 1094, 1920, Chem. Abs. 1920, 3267.

[24] **McClendon, Cole, Engstrand,** and **Middlekauf,** J. Biol. Chem. 40, 243, 1919.

[25] **Jackson** and **Harley,** Lanc. 1900, i. 1184, Proc. Roy. Soc. 1900, 66, 250.

[26] **Holst** and **Frölich,** Z. f. Hyg. infekt. 72, 1, 1912.

The theory of bacterial origin [27] is refuted by the work of **Givens and Hoffman** [28] who found that the blood of scorbutic guinea pigs is invariably sterile, regardless of the diet on which the disease is produced. No marked difference was found in the intestinal flora when smears and cultures were made from different parts of the intestinal

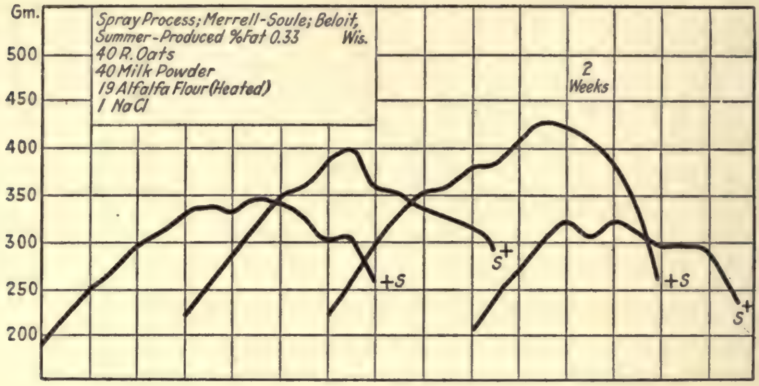

FIG. 10

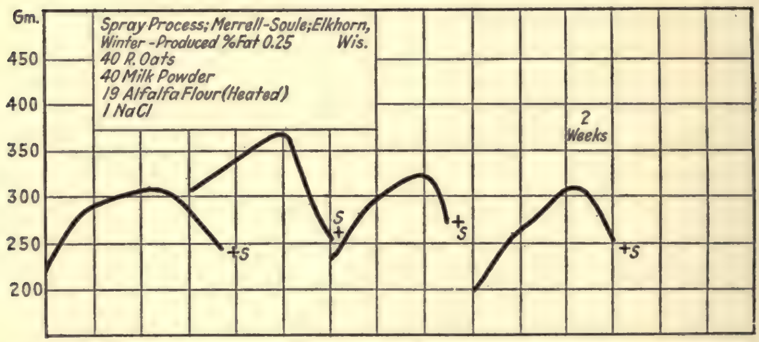

FIG. 11.—The upper chart shows the failure of skimmilk powder from summer-produced milk to protect against scurvy. The lower chart indicates a similar failure when using winter-produced milk.

Courtesy of Messrs. E. B. Hart, H. Steenbock, N. R. Ellis and Journal of Biological Chemistry (46, 311, 1921).

[27] **Coplans,** Tr. Epidemiol. Soc. 23, i, 1904; **Jackson and Moody,** J. Infect. Dis. 19, 511, 1916.

[28] **Givens and Hoffman,** Proc. Soc. Biol. Chem. J. Biol. Chem. 41, xxxiii, 1920.

tract of guinea pigs on a diet of oats alone, on oats plus 3 cc. of lemon juice daily, after scurvy developed, on soy cake diet, and on the same plus cabbage after the appearance of scurvy. The observations recorded as to the presence of bacterial infection in certain cases of

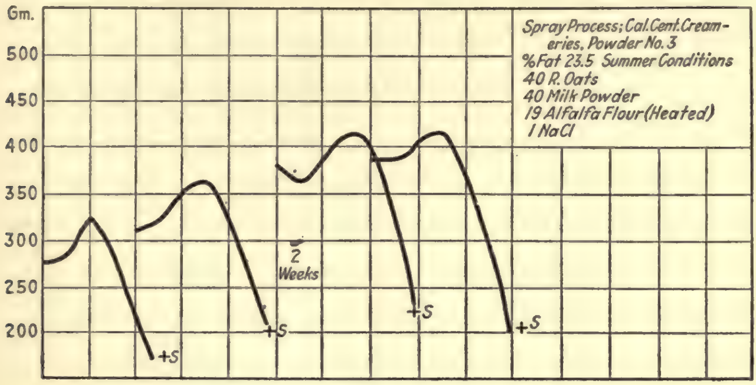

FIG. 12

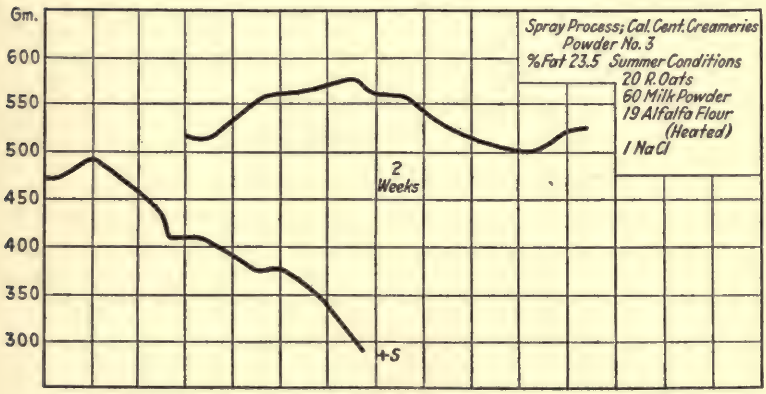

FIG. 13.—The upper chart shows the failure of a whole milk powder made by a spray process to protect against scurvy. An improvement is shown in the lower chart which records the results of feeding a larger proportion of the same milk powder. This powder still retained some of the antiscorbutic vitamin but not enough, even at this high level of feeding, to protect all individuals.

Courtesy of Messrs. E. B. Hart, H. Steenbock, N. R. Ellis and Journal of Biological Chemistry (46, 314, 1921).

scurvy can easily be explained by the susceptibility of animals in a poor condition of health to such infection.[29] The faulty intestinal

[29] See **McCollum** and **Pitz**, J. Biol. Chem. 31, 233, 1917; **Abels**, Wien. klin. Woch. 33, 899, 1920.

condition referred to by **Steenbock**[30] is also doubtless a result rather than the cause of the disease, and the same may be said of inanition.[31]

The theory of **McCollum** and **Pitz**[32] relating scurvy with chronic constipation has been disproved by numerous investigators. The argument is summed up by **Cohen** and **Mendel.**[33]

It is highly improbable, they observe, that constipation is the predisposing cause of scurvy, as can be seen from the results in feeding milk. A small amount ingested daily permitted the onset of symptoms of scurvy, while large quantities, which were not less constipating, caused the scorbutic symptoms to disappear. Moreover, aids to the ready elimination of feces, like differing amounts and kinds of roughage caused no corresponding change in the course of the disease. Contrary to the findings of **McCollum** and **Pitz,** other investigators, namely **Hess** and **Unger,**[34] were unable to protect or cure animals with

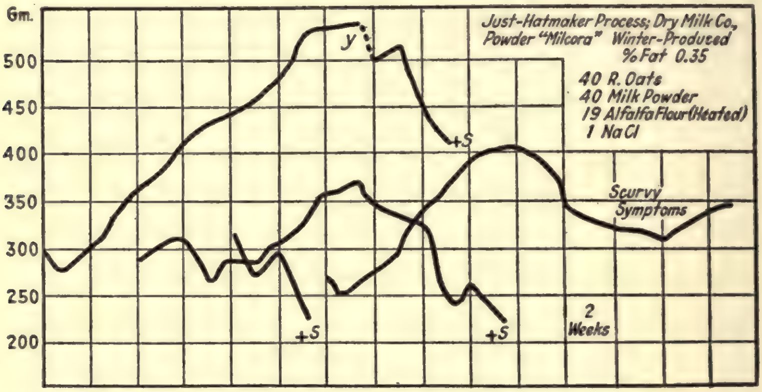

FIG. 14.—This winter-produced skimmed milk, converted to a powder by a roll drying process, had lost in a considerable measure its antiscorbutic properties. In most cases the onset of scurvy was greatly delayed. The powder contained 0.35 per cent of fat.

Courtesy of Messrs. E. B. Hart, H. Steenbock, N. R. Ellis and Journal of Biological Chemistry (46, 317, 1921).

daily additions of mineral oil to a scurvy-producing diet. Neither were they able to induce active scurvy in a latent case by the production of marked constipation with morphine. Recently Hess and Unger reported the cure of scurvy by means of intra-venous injections of orange juice. This successful antiscorbutic effect apparently was not due to specific relief from constipation or its attendant intestinal complications.

On the other hand, one must not forget that marked scorbutic symptoms

[30] Sci. Mo. 7, 179, 1918.

[31] **Furst,** Z. Hyg. 72, 121, 1912; **Lewis** and **Karr,** J. Biol. Chem. 28. 17, 1916-17; **Cohen** and **Mendel,** J. Biol. Chem. 35, 430, 1918.

[32] **McCollum** and **Pitz,** J. Biol. Chem. 31, 229, 1917; **Pitz,** *Ib.*, 33, 471, 1918.

[33] **Cohen** and **Mendel,** J. Biol. Chem. 35, 425, 1918.

[34] **Hess** and **Unger,** Proc. Soc. Exp. Biol. Med. 15, 82, 1918.

have been induced in guinea pigs on laxative diets. Infantile scurvy has also been found to occur quite as often in connection with diarrhea as with constipation.[35] It seems clear, therefore, from the foregoing facts that constipation is not the causative factor in scurvy. Undoubtedly the vital resistance of a scorbutic animal is lowered, thus rendering it more susceptible to unfavorable conditions like infections and toxemias.

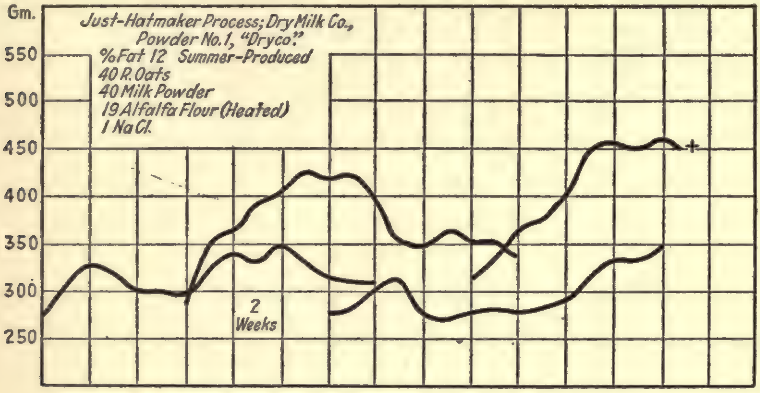

FIG. 15

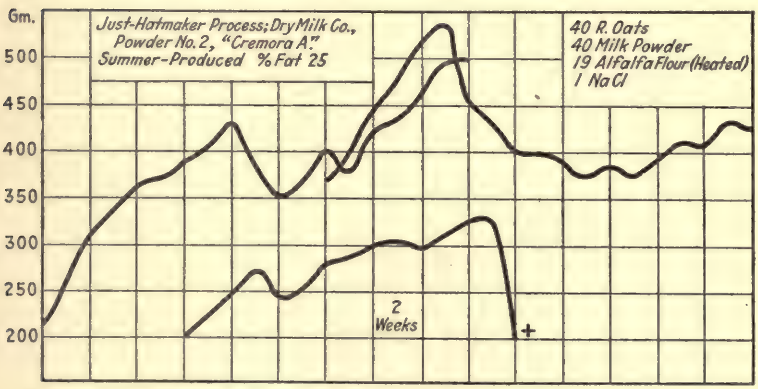

FIG. 16.—Adequate protection against scurvy was offered by these milk powders containing, respectively, 12 per cent and 25 per cent of fat. The powders were made by a roll drying process.

Courtesy of Messrs. E. B. Hart, H. Steenbock, N. R. Ellis and Journal of Biological Chemistry (46, 315, 1921).

Gerstenberger[36] sought for a connection between scurvy and derangement of calcium metabolism.

[35] Cf. American Pediatric Society Committee, Arch. Pediat. 1898, 15, 481.
[36] **Gerstenberger**, Amer. J. Med. Sc. 145, 253, 1918.

In view of the known data regarding the pathology, chemistry, and symptomatology of scurvy on the one hand, and regarding the important function played by calcium in bone growth, nerve conduction, vessel sealing, and cell permeability on the other, **Gerstenberger** considered that all of these different system symptoms might be explained on the common basis of an interference with one or more of the normal functions of calcium and its physiological anion in the osseous system, vascular system, nervous system and probably other systems. That scurvy is not a calcium deficiency disease is indicated by the increase in the calcification at **Frankel's** line but while there is an excess of calcium deposited at one part of the bone there is nevertheless in all probability not so much calcium in the bone as a whole in advanced stages of scurvy as there would be in the normal bone, because the rate of growth of the bone is much less rapid than under normal conditions, while the normal process of resorption is going on, producing a rarefication and brittleness with consequent loss of material. He fed cod liver oil containing tricalcium phosphate to scorbutic animals, but without result. Similar ill-success attended the administration of calcium in different forms by **Holst** and **Frölich**.[37]

Pitz[38] was able to protect guinea pigs against scurvy for 18 weeks by addition of meat and tricalcium phosphate to a ration of rolled oats and milk. He found some beneficial effect from the ingestion of tricalcium phosphate alone, but not so pronounced as when meat was introduced. Calcium chloride was more effective than tricalcium phosphate, showing that the phosphorus is not of prime importance. When sodium chloride was added to the oats, milk, meat, and tricalcium phosphate ration the onset of scurvy was delayed still further. **Pitz** concludes from this that the chlorine metabolism is deranged in scurvy as well as the calcium metabolism, and that the added calcium salts afford a certain amount of protection by controlling the permeability of the tissues of the animal and thereby affording protection against invading agents.

Pugliese[39] asserts that the conditions produced in animals by a ration of dried grain are not dependent upon the absence of the antiscorbutic vitamin, but are caused by a change in the reaction of the organic fluids, and a resultant demineralization of the organism, especially with respect to phosphorus and calcium.[39a]

[37] **Holst** and **Frölich**, Zeitsch. Hyg. Infekt. 72, 1, 1912.

Young guinea pigs weighing 250–300 g., fed on a diet deficient in water-soluble *C*, show at death a pronounced increase in weight of the adrenal glands; this may indicate a compensatory response to the decreased adrenaline production known to exist in the scorbutic animal. The heart and kidneys appear to be increased in weight on scorbutic diets, while the liver shows no effect, **LaMer** and **Campbell** (Proc. Soc. Exptl. Biol. Med. **18**, 32, 1920).

[38] **Pitz**, J. Biol. Chem. 36, 439, 1918.

[39] **Pugliese**, Rend. r. Inst. Lomb. Sci. Lett. 52, 723, 1919; Physiol, Abstracts 5, 92, 1920.

[39a] **Findlay** (Biochem. J. 15, 355, 1921) claims that lack of vitamin *A* is in no way responsible for the lesions that occur in experimental scurvy.

Howard and **Ingvaldsen**[40] studied the mineral metabolism of the monkey during experimental scurvy, but without significant result. They state that the changes in the mineral excretion of the monkey during the scorbutic period are not sufficiently significant to admit of easy interpretation. The marked loss of the various mineral substances encountered in previous experiments with man and guinea pigs not having been observed in the present series. These investigators are of the opinion that a study of the intake and output of the inorganic elements in human adult scurvy and the experimental scurvy of the guinea pig and the monkey does not yield sufficiently decisive information to warrant an explanation of the pathogenesis of scurvy.

Robb[41] found that guinea pigs subsisting on a diet of dried plants 14 or 21 days before the experimental period, eliminated twice as much calcium as those that had been on a green diet. Scurvy appeared in all animals.

While there is either lack of confirmation of or positive evidence against each theory of the origin of scurvy except that of deficiency in diet, there is convincing evidence in favor of this in the history of the disease and its treatment. As early as the middle of the Eighteenth Century **Lind** published an account of the disease showing the remarkable curative power of orange and lemon juice.[42] This quaint report is of interest from a historical standpoint:

On the 20th of May, 1747, I took twelve patients in the scurvy, on board the "Salisbury" at sea. Their cases were as similar as I could have them. They all in general had putrid gums, the spots and lassitude, with weakness of their knees. They lay together in one place, being a proper apartment for the sick in the fore-hold; and had one diet common to all, viz. water-gruel sweetened with sugar in the morning, fresh mutton-broth oftentimes for dinner; at other times light puddings; boiled biscuit with sugar, etc., and for supper, barley and raisins, rice and currants, sago and wine, or the like.

Two of these were ordered each a quart of cider a day. Two others took twenty-five drops of elixir vitriol, three times a day, upon an empty stomach; using a gargle strongly acidulated with it for their mouths. Two others took two spoonfuls of vinegar three times a day upon an empty stomach; having their gruels and their other food well acidulated with it, as also the gargle for their mouths. Two of the worst patients, with the tendons under the ham rigid (a symptom none of the rest had) were put under a course of sea-water. Of this they drank half a pint every day, and sometimes more or less, as it operated by way of gentle physic. Two others had each two oranges and one lemon given them every day. These they ate with greediness, at different times upon an empty stomach. They continued but six days under this course, hav-

[40] Howard and Ingvaldsen, Bull. Johns Hopkins Hosp. 28, 222, 1917.

[41] Robb, Science 52, 510, 1920.

[42] Lind, Treatise on Scurvy, London, 2nd Ed. 1757.

ing consumed the quantity that could be spared. The two remaining patients took the bigness of a nutmeg three times a day of an electary recommended by a hospital-surgeon, made of garlic, mustard-seed, rad. raphan, balsam of Peru, and gum myrrh; using for common drink, barley water well acidulated with tamarinds; by a decoction of which, with the addition of cremor-tartar. they were gently purged three or four times during the course.

The consequence was, that the most sudden and visible good effects were perceived from the use of the oranges and lemons; one of those who had taken them being at the end of six days fit for duty. The spots were not indeed quite off his body, nor his gums sound; but without any other medicine than a gargarism of elixir vitriol, he became quite healthy before we came into Plymouth, which was on the 16th of June. The other was the best recovered of any in his condition; and being now deemed pretty well, was appointed nurse to the rest of the sick.

Next to the oranges, I thought the cider had the best effects. It was indeed not very sound, being inclinable to be aigre or pricked. However, those who had taken it were in a fairer way of recovery than the others at the end of the fortnight, which was the length of the time all these different courses were continued, except the oranges. The putrefaction of their gums, but especially their lassitude and weakness, were somewhat abated, and their appetite increased by it.

The regular administration of lemon juice prescribed by the British Admirality in 1780 and the similar regulation adopted by the Board of Trade in 1865 has resulted in the complete disappearance of the disease from the navy and mercantile marine. Whereas in 1780 the number of cases of scurvy received into Haslar Hospital (a purely naval hospital) was 1457, there was only one case in 1806 and one more the following year. Dr. **Baly**[43] states that in several prisons the occurrence of scurvy wholly ceased on addition of a few pounds of potatoes being made to the weekly diet. **Holst**[44] asserts that when the Eskimos suffer from this disease they turn to the liver of seals or better to fresh "matok," a preparation from the skin of whales, for cure.

While the experience of generations has pointed to fresh fruits and vegetables as the preventive and cure of scurvy, only after the discovery of **Holst** and **Frölich**,[45] that the disease could be induced in guinea pigs by restricting them to a diet of oats and water and that the symptoms thus produced responded to the same treatment which had been found effective in human scurvy, did it become possible to classify foods into those which do and those which do not possess antiscorbutic power.[46] Tradition has placed the juice of lemons and

[43] 1814-61, quoted by the University Encyclopedia.

[44] Holst, "15th International Cong. of Hyg." Wash., 1912, ii, 588.

[45] Holst and Frölich, J. Hyg. 7, 634, 1907; Z. f. Hyg. Infekt. 72, 1, 1912.

[46] For detailed classification see Chap. 4 and Appendix. Distribution of *C*.

Fig. 17. — A normal guinea pig. 15 c.c. daily of summer pasture milk protected against scurvy when the remainder of the ration consisted of heated alfalfa flour 25 parts, rolled oats 74 parts, and common salt 1 part. Photographed in the 12th week of restriction to this diet.

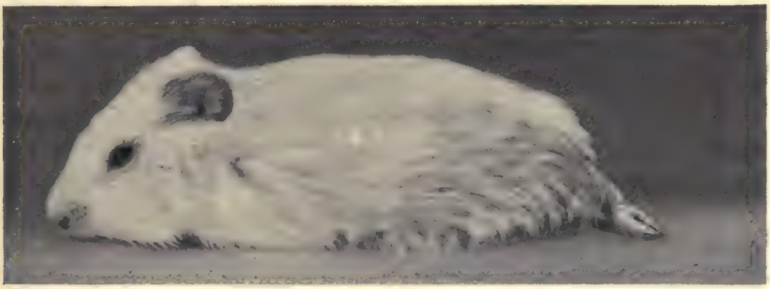

Fig. 18. — A scorbutic guinea pig. 15 c.c. daily of dry feed milk did not protect against scurvy when the remainder of the ration consisted of heated alfalfa flour 25 parts, rolled oats 74 parts, and common salt 1 part. Photographed after 4 weeks' restriction to this diet.

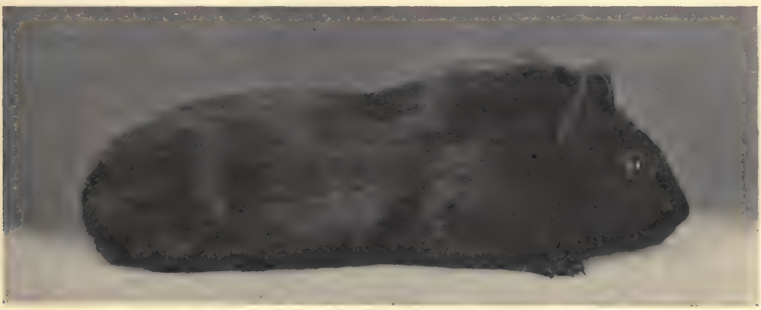

Fig. 19. — Showing early stages of scurvy. 15 c.c. daily of winter-produced milk (silage + dry grain and dry hay) did not effectively protect against scurvy when the remainder of the ration consisted of heated alfalfa flour 25 parts, rolled oats 74 parts, and common salt 1 part. Photographed after 4 weeks' restriction to the diet. The silage was made from field-dried, but non-frosted corn.

Figs. 17, 18 and 19 supplied through the courtesy of Messrs. E. B. Hart, H. Steenbock, N. R. Ellis and Journal of Biological Chemistry 42, 396, 1920.

oranges first among the curative substances, and modern research largely confirms this view, although certain other vegetable products have been found to be equally efficacious. Tomatoes, raw or canned, the uncooked juice of the Swedish turnip, leafy vegetables, especially cabbage, eaten raw, the sprouted seeds of cereals, peas, or beans are all efficient antiscorbutics. It is an interesting fact that the swedish turnip, which might at first sight appear to be quite unrelated to the other classes of vegetables named, is really a member of the order Cruciferae, to which belong the cabbage and the "scurvy-grass" (*Cochlearia officinalis*) much esteemed in olden days as a cure for the disease. **Bachstrom,** in 1734, told how a sailor in the Greenland ships, so disabled with scurvy that he could only crawl about the ground, was put ashore by his companions and left to perish. In his desperate state he nibbled the grass growing on the shore, which chanced to be scurvy grass, and in a short time recovered so that he was able to find his way home.[47]

The minimum daily doses of fresh citrus fruit juices required to protect a guinea pig from scurvy were found by **Davey** (Biochem. J. **15**, 83, **1921**) to be as follows: Lemon 1.5 cc., orange 1.5 cc. and lime 5 cc. To avoid the acid effects of these juices they were first partially neutralized with sodium carbonate.

Animal food, while of undoubted value, is less effective than vegetable products. Cow's milk contains comparatively little of the necessary factor, especially after pasteurization. Fresh meat is effective if eaten in sufficiently large quantities, but the necessary cooking lessens its value. In general, heating and drying decrease the antiscorbutic effect of foodstuffs, the deterioration being proportional to the time of exposure, but some foods, for instance, tomatoes, withstand such unfavorable conditions better than others. **Chick** and **Dalyell**[48] have reported many cases of scurvy developing among children who were under treatment for tuberculosis and consequently on a diet rich in antiscorbutic foodstuffs. Investigation showed that these foods had been subjected to prolonged cooking in the course of which the antiscorbutic properties had evidently been completely destroyed. Similar cases of a theoretically adequate diet being rendered scorbutic by over-cooking of the food occurred from time to time in the armies during the war.

It is to be remembered that our data as to the antiscorbutic efficiency of different foods is in large measure derived from guinea

[47] Quoted in Rep. 38, Med. Res. Com. London, 1919.

[48] **Chick** and **Dalyell,** Z. Kinderheil. **26**, 257, 1920.

requirement for this factor it is quite possible that certain foods which would appear from these experiments to be entirely devoid of antiscorbutic vitamin may actually contain an amount which would afford sufficient protection to a less susceptible animal. For practical purposes, however, the guinea pig test is quite satisfactory, since it serves to distinguish those foods which are relatively high from those which are at least relatively low in antiscorbutic content.

CHAPTER XIII

XEROPHTHALMIA

Experimenters in animal nutrition have frequently observed the susceptibility of animals on certain deficient diets to an affection of the eye which is variously described as a conjunctivitis,[1] a xerophthalmia [2] or a keratomalacia.[3] This usually begins with swelling of the eyelids, followed by an inflammation of the conjunctivitae and a haemorrhagic discharge which frequently becomes purulent. Unless treated the cornea eventually becomes involved and total blindness results.

Wason [4] sums up the results of a study of the pathology of this eye condition as follows:

1. The primary etiological factor in ophthalmia of rats is lack of the vitamin *A*.
2. The nature and mechanism of the change in these rats whereby their corneas are rendered susceptible to bacterial invasion is unknown.
3. The type and virulence of the organism of secondary infection determine, in part at least, the course of the disease.
4. The anatomic manifestations of the disease are characterized by hyalinization or necrosis of the outer layer of the corneal epithelium, exudation of serum and cells into epithelium and stroma, and proliferation of the blood vessels and fibroblasts. In advanced cases invasion of the anterior, and occasionally of the posterior chamber result.
5. The degree to which restoration is possible depends on the extent of the secondary injury.

While this disease has been most commonly reported in rats, **Nelson** and **Lamb** [5] have found that rabbits are also susceptible, although they failed to produce the characteristics in guinea pigs or

[1] **Falta** and **Noeggerath,** Beit. 3, Chem. Physiol. u. Path. 7, 313, 1906. **Knapp,** Z. f. Exp. Path. 5, 147, 1908; **Steenbock,** Sci. Mo. 7, 179, 1918.

[2] **McCollum** and **Simmonds,** J. Biol. Chem. 32, 182, 1917.

[3] **Stevenson** and **Clark,** Bioch. J. 14, 502, 1920.

[4] **Wason,** J. Am. Med. Ass. 76, 908, 1921.

[5] **Nelson** and **Lamb,** Sci. 52, 393, 566, 1920.

chickens under the same conditions. **Guerrero** and **Concepcion**[6] however, report cases of xerophthalmia developing in fowls fed on a polished rice ration. Of five birds on an exclusive diet of polished rice all developed polyneuritis and one became blind. Of 30 more which were given polished rice with the addition of "tikitiki extract" five developed polyneuritis and seven died after developing xerophthalmia, which appeared in some cases as early as the seventh day and in others not until the seventy-ninth day of the experiment.

Symptoms similar to those noted in animals have been described in young children whose diet is known to have been deficient in fat, **Mori**[7] reported over 1400 cases of such a disease in Japanese children, which he believed to be due to fat starvation and which was cured by administration of liver (chicken and fish) or eel fat. **Czerny** and **Keller**[8] describe what are probably analogous cases of eye disease in children suffering from malnutrition as a result of restriction to a cereal diet, and further instances, all of which appear to be of dietary origin, are given by **Monrad**[9] and **Ronne**[10] and **Bloch.**[11] **Bloch** describes over fifty cases observed in Copenhagen. The most severe cases occurred in children about twelve months old, who had been fed on separator milk, and were characterized by hardening of the cornea, ulceration, necrosis, and ultimate blindness. External treatment gave little relief and no permanent improvement. As all the patients showed pronounced symptoms of malnutrition Bloch was led to connect the eye disease with this general condition, and investigation into their previous diets suggested that the most serious defect in their nutrition was lack of fat. The treatment consisted in feeding on whole milk together with as much cod liver oil as could be tolerated, which resulted in the rapid disappearance of the eye trouble together with marked general improvement. On milk alone, without the addition of the cod-liver oil, recovery was much slower, and was frequently too late to save the sight.

A particularly interesting case noted was that of an orphanage in which there were 86 children of various ages up to two years. The inmates were divided into three sections, group I, made up of infants and ailing children, and two groups, IIa and IIb, of older children. Eight cases of xerophthalmia appeared in group IIa, while

[6] **Guerrero and Concepcion,** Phil. J. Sci. 17, 99, 1920.

[7] **Mori,** Jahrb. f. Kinderh. 59, 175, 1904.

[8] **Czerny and Keller,** Des Kindes, Leipsic. 1906, pt. 2, p. 67.

[9] **Monrad,** Ugesk. f. Laeger, 79, 1177, 1917.

[10] **Ronne,** Ugesk. f. Laeger, 79, 1479, 1917.

[11] **Bloch,** Ugesk. f. Laeger, 79, 349, 1917; Rigshosp. Boerneaf. Med. 2, 1, 17; 3, 57, 1918. Quoted in Rep. 38, Med. Res. Com. p. 76.

the rest of the institution was free from it. Group I was receiving the whole milk, on which they all made satisfactory progress. Group II received an apparently liberal diet of cocoa, buttermilk, milk foods made with separator milk, various kinds of bread, margarine, cereals, fruit syrups, boiled fish, minced meat, and mashed potatoes. No butter, eggs, or cream was used, and no whole milk except a small amount which was given to group IIb with a beer and bread porridge for breakfast. The children in IIa (the group in which the xerophthalmia appeared) breakfasted off rusks and gruel and were given no whole milk. As this small addition of whole milk to the diet of one group was the only variation in the treatment of the older children it would appear that it must have been sufficient to prevent the onset of the disease in group IIb. Local treatments have failed to cure the eye condition (although it was slight in all cases.) Ten gms. of cod-liver oil were administered twice a day, resulting in a complete cure in eight days. Thereafter the separator milk in the diet was partly replaced by whole milk.

On the other hand, **von Gröer** [12] succeeded in maintaining two infants during their first half year of life on a practically fat-free diet, consisting at first of separator milk and sugar alone, and later of a porridge made with skimmed milk and sugar. The children grew at practically normal rate, and no symptoms of eye disease are reported, although there are some indications of lowered nutritive condition. Still more striking is the study reported by **Hess** [13] in which five infants varying in age from five to twelve months were fed for eight or nine months on a diet consisting of 180 gms. of highly skimmed milk ("Krystalak," 0.2 per cent fat), 30 gms. cane sugar, 15 to 30 gms. autolyzed yeast, 15 c.c. of orange juice, 30 gms. of cottonseed oil, and cereal for the older infants. On this diet they are reported to have done well and to have shown no signs of eye trouble.

While these results indicate that a fat deficiency may be withstood for a comparatively long period without obvious injury especially in early infancy, it may still be contended that prolonged feeding on such diets may be responsible for the onset of the disease in question.

That the fats themselves are not the influential factor is indicated by the cases referred to in which the disease developed on a diet containing a moderate amount at least of margarine, and finds confirmation in the work of **Osborne** and **Mendel** [14] in which they demonstrated that rats could be maintained in excellent nutritive

[12] **Von Gröer,** Bioch. Z. 97, 311, 1919.

[13] **Hess,** Proc. Am. Soc. Biol. Chem., J. Biol. Chem. 41, xxxii, 1920.

[14] **Osborne and Mendel,** J. Biol. Chem. 45, 145, 1920.

condition over considerable periods of time on diets practically free from fats provided they were supplied with adequate amounts of the vitamin *A*.[15] If *A* is lacking in the ration on the other hand, the disease almost invariably makes its appearance among rats, even though the diet be amply supplied with fats such as lard and cotton seed oil.[16]

While there is general agreement as to the close connection of this disease with deficiency of *A*, there is some difference of opinion as to whether the eye-trouble is the direct outcome of the deficiency or is an infection which is successfully resisted by the normal animal but which develops and spreads rapidly when the resistance is lowered by malnutrition. **Hopkins** and **Chick**[17] state that animals deprived of *A* become highly susceptible to bacterial infection.

In the case of rats they note that this lowered resistance first becomes apparent in many cases by the appearance of a characteristic infection of the external eye, which has been provisionally classified as a xerophthalmia. **Osborne** and **Mendel**[18] remark: "Another type of nutritive deficiency exemplified in a form of infectious eye-disease prevalent in animals inappropriately fed is speedily alleviated by the introduction of butter-fat into the experimental rations."

Hess, McCann and **Pappenheimer**[18a] carried out some tests involving feeding young rats a diet markedly deficient in fat-soluble *A*. The diet consisted of casein, rice starch, salt mixture and, as fatty material, Crisco was used. In one case yeast also was administered and in another series of tests the animals were fed orange juice. A control set of rats received the same diet except a portion of the Crisco was replaced by 6% of butter-fat. They found that young rats receiving the diet, complete except for lack of fat-soluble *A*, invariably failed to grow and generally developed keratitis.

Bulley[19] kept about 500 young rats on experimental diets for over a year, 50 per cent having an adequate amount of *A*, and the remainder on diets in which *A* was either deficient or absent (as shown

[15] See also **Drummond**, Bioch. J. 13, 95, 1919.

[16] **Goldschmidt**, Arch. f. Ophth. 90, 354, 1915; **Freise, Goldschmidt,** and **Frank,** Monatschr. f. Kinderh. 13, 424, 1915; **Halliburton** and **Drummond**, J. Physiol. 51, 235, 1917; **Steenbock, Boutwell,** and **Kent,** J. Biol. Chem. 35, 517, 1918; **Steenbock** and **Gross,** 26, 40, 501, 1919; **Steenbock** and **Boutwell,** *Ib.*, 41, 163, 1920; 42, 131, 1920; **Steenbock,** Sci. 50, 352, 1919; **Emmett** and **Sturtevant,** Sci. 52, 390, 1920.

[17] **Hopkins** and **Chick,** Rep. 38, Med. Res. Com. p. 17.

[18] **Osborne** and **Mendel,** J. Biol. Chem. 16, 431, 1913.

[18a] **Hess, McCann** and **Pappenheimer,** J. Biol. Chem. 47, 395, 1921.

[19] **Bulley,** Bioch. J. 13, 103, 1919.

by failure to grow). All were given fat-free alcoholic extract of yeast preparation as a source of *B*. During the whole period only five cases of xerophthalmia developed, of which two occurred on a diet which contained a limited supply of *A;* one occurred on a diet in which there was presumably sufficient *A;* one on a diet in which there was no *A;* and one on the ample stock diet. Two of the animals were growing well at the time, one remained with weight unchanged, and one lost weight. Each experiment lasted three or four months, and in each at least eight other rats were used which showed no sign of xerophthalmia. **Bulley** also reports unpublished experiments carried on by **Totani,** who kept rats for many weeks on a diet of extracted starch, extracted casein, sugar, lard, and the artificial salt mixture of **Osborne** and **Mendel,** without any signs of xerophthalmia, although **Hopkins** had previously found that this identical diet brought about typical xerophthalmia within fourteen days. **Bulley** believes therefore that xerophthalmia is an infection, and that improvement on addition of *A* to the diet is simply the result of general improvement in health.

Stephenson and **Clark** [20] ascribe **Bulley's** results to lack of sufficient purification of the basal diet. They found that among 46 rats, on a diet defficient in *A,* 28 per cent developed the typical eye-trouble, which was cured in every case by administration of *A.*

Against the theory of infection is the fact that the disease cannot be transmitted from one animal to another.[21] Moreover, although absence of any one of the essential factors from the diet results in malnutrition, the xerophthalmia referred to is generally regarded as specific to deficiency of *A.* **Steenbock** [22] states that an indistinguishable form sometimes occurs in animals on other rations,[23] a theory which would serve to explain the observations of **Funk** and **Macallum.** These observers [24] found that rats fed on a yeast and butter diet often exhibit the eye affections regarded by most investigators as characteristic of dietary deficiencies. **Macallum** [25] noted that the substitution of moist brewers yeast for the dried form relieved the eye trouble to such an extent that it could be easily controlled by the use of dilute sulphate solutions, and **Funk** and **Dubin** [26] have

[20] **Stevenson** and **Clark,** Bioch. J. 14, 502, 1920.

[21] **Emmett,** Sci. 52, 157, 1920; **Nelson and Lamb,** *Ib.,* 566; Am. J. Physiol. 51, 530, 1920.

[22] **Steenbock,** Sci. Mo. 7, 179, 1918.

[23] See also **Steenbock and Boutwell,** J. Biol. Chem. 41, 90, 1920; **Bulley,** Bioch. J. 13, 103, 1919.

[24] **Funk and Macallum,** J. Biol. Chem. 27, 51, 1916.

[25] **Macallum,** Tr. Roy. Can. Instit., 1919, p. 175.

[26] **Funk and Dubin,** Sci. 52, 448, 1920.

reported that out of 30 rats on artificial diets only one developed keratomalacia, and this was one which was getting five per cent of cod liver oil in the ration. In this case the eye condition was cured by the administration of about 2 cc. of autolyzed yeast per day.

McCollum [27] regards xerophthalmia as a true deficiency disease specifically due to a lack of *A*. His view is confirmed by **Emmett** [28] who reports that out of 122 rats in a group fed on a diet in which *A* was lacking, 120 (98.3 per cent) showed positive signs of xerophthalmia sooner or later, and when *A* was present, with or without *B*, none of the 319 rats under observation showed evidence of this trouble. All the rats were fed individually in practically every case. They were kept in metal cages without any bedding, provided with a special removable wire screen floor. The cages and the food and water cups were always disinfected once or twice a week. The conditions were precisely the same for all the rats and the possibilities of infection were uniform.

Osborne and **Mendel** [29] have reviewed the statistics on 1000 rats from their own stock to which no animals have been admitted from extraneous sources for several years.

All of these animals have been under study for at least a year. They are kept in the same laboratory under conditions which are identical except as to diet. Their cages are sterilized twice a week, but the rats are regularly weighed in a common container, and it is stated that the proximity of the open-mesh wire cages to one another offered sufficient opportunity for dissemination of contagion. Nevertheless, in observations on several thousands of rats they have never observed the distinctive symptoms of eye disease in any animals except those suffering from deficiency of *A*. The results for the particular group under consideration are summed up as follows:

	Total number	Number with eye trouble
On diets deficient in *A*	136	69 (50%)
" " " " *B*	225	0
" " " " other factors	90	0
" " experimental but presumably adequate	201	0
" mixed food (stock diet)	348	0
	1000	69

In only a few cases did administration of *A* fail to effect a cure. In a number of cases a dosage of 100 mg. of butter-fat per day effected a cure within a week, and in others 42 mg. of grass oil per day relieved the symptoms within

[27] **McCollum** and **Simmonds**, J. Biol. Chem. 32, 182, 1917; **McCollum, Simmonds,** and **Parsons,** *Ib*. 38, 124, 1919.

[28] **Emmett,** Sci. 52, 157, 1920.

[29] **Osborne** and **Mendel,** J. Am. Med. Ass. 76, 905, 1921.

10 days, 70 mg. of alfalfa oil within seven days, and 66 mg. of spinach oil within 21 days. They point out that the xerophthalmia is rare in more mature animals even on diets free from *A*. **Freise, Goldschmidt** and **Frank** (l. c.) could not observe it in rats which weighed more than 120 g. when the experiments started. **Osborne** and **Mendel,** however, have occasionally observed it even in large animals.

McCollum [30] emphasizes one point which may help to explain some of the apparently contradictory results of different observers; namely, that an amount of *A* which is amply sufficient when the diet is otherwise well constituted will prove inadequate in other cases where the quality of one or another factor is of a low order. In the case of

Fig. 20.— This child was fed on skimmed milk and as a result an ulcer developed on one eyeball. The remedy is the use of plenty of cream, butter or cod liver oil.

Courtesy of the Connecticut Agrciultural Experiment Station (Bulletin 215, Photograph from Bloch).

animals on a diet consisting principally of seed products it has been found that xerophthalmia develops unless additional *A* is supplied. If, however, the seed ration is supplemented by additional protein and inorganic salts, in both of which seeds are deficient, the animals may withstand the onset of xerophthalmia for a long time, even

[30] **McCollum,** "Newer Knowledge of Nutrition," p. 87; **McCollum** and **Simmonds,** J. Biol. Chem. 32, 347, 1917.

though no supplementary *A* is given, whereas if the deficiency is made up in respect to one factor only, for instance inorganic salts, the combined effects of lack of two factors simultaneously (in this case protein and *A*) will hasten the onset of xerophthalmia.

When judging the effects of the diet on an animal, **McCollum** advocates the precaution to take into account the fact that the diet is a complex thing, and that if it is properly constituted with respect to all factors but one an animal may tolerate it without apparent injury whether the fault lies in one or another of the essential components. Hence the value of one component may fall well below that which will lead to serious malnutrition, when a second dietary factor is likewise inadequate.

The suggestion has been made occasionally that " night blindness " is a deficiency disease occurring in adults but related to the xerophthalmia of children and animals on a diet deficient in *A*. The observation that mature animals are very little susceptible to eye trouble as a result of such deficiency would cast some doubt on this theory. More convincing, however, are the observations made by **Appleton** in the course of a study of the diet in Labrador,[31] from which the conclusion is reached that night blindness is a negative after-image following long exposure to the dazzling glare of sun on snow. A poor diet may increase the probability of seeing after-images just as fatigue does. The disease occurs almost exclusively among men in Labrador, and yields readily to any treatment. Only two cases could be found to have occurred among women.

[31] **Appleton,** J. Home Ec. 13, 199, 1921.

CHAPTER XIV

PELLAGRA

WHETHER pellagra is or is not to be regarded as one of the deficiency diseases from the point of view of the vitamin hypothesis is still open to question. There is, however, sufficient evidence for the affirmative to warrant its consideration at least.[1]

The disease is one which has been common for centuries in some parts of Europe, notably in Northern Italy and Roumania. It made its appearance in Southern France in the disastrous period following the reign of Napoleon, but disappeared later, apparently with the betterment of economic conditions, and is now almost unknown there. While there are records of occasional cases in the Southern United States as far back as 1828,[1a] it was little known until about the beginning of the present century. In the course of the last twenty years it has increased rapidly, no fewer than 500,000 cases having been reported within this period. The Metropolitan Life Insurance Company, which operates extensively in the cities and towns of the South, records 2,310 deaths from this disease between 1911 and 1916, a death rate which is higher than that for either malaria or general tuberculosis. In 1916 pellagra ranked fourth as a cause of death in Mississippi, with 840 deaths, third in Alabama, with 677 deaths, and second in South Carolina, with 627 deaths. At the time of the second Pan American Scientific Congress it was said to be practically unknown in Central and South America, but **Roberts** has recently stated

[1] **Babes** (Bull. soc. sci. acad. Roumaine 6, 138, 1920) considers that recent researches have not been of a nature to clarify either the etiology or the pathogenesis of pellagra. He thinks they have only increased the uncertainty by new hypotheses, by misinterpretation of facts, and by poorly executed work which will pass into oblivion. The following facts concerning pellagra he avers have been established: (1) The close relationship of the disease with the eating of maize, especially unwholesome maize. (2) The predisposition produced in inanition, weakness, congenital deterioration especially of a nervous type, certain diseases, and convalescence from these diseases. (3) The specific nature of pellagra. (4) The favorable action of an abundant mixed diet and the administration of atoxyl.

[1a] **Goldberger,** Proc. 2nd, Pan. Am. Sci. Cong. 1917, Section 8, Pt. 2, p. 20.

that it is now found in Mexico, Yucatan, Panama, and the Hawaiian Islands, as well as in every state in the Union.

It is very commonly asserted that pellagra attacks the white race more frequently than the negroes, but while this is true in certain sections of the country, in others the reverse holds. In Charleston, S. C. there are said to be three cases among the colored population to every one among the whites, while in Alabama the disease is evenly distributed among the negroes and the whites.[2]

It has very rarely been observed in children under the age of one year, but is not so rare in the second year and fairly common between two and twelve years. The period from twelve to sixteen years is relatively free from initial attacks of pellagra, but after sixteen, pellagra incidence rises rapidly in women, the rise being especially sharp in colored women. It is very much more common among women than among men. In a group of 414 cases between the ages of 20 and 44 coming under the observation of the Thompson Pellagra Commission there were 357 women as against 57 men.[3] Between the ages of 20 and 50 years, however, the number of women attacked gradually diminishes and the number of men gradually increases, so that the two sexes are approximately equal in this respect at 50. In old age the onset of pellagra is slightly more common in men than in women.

The disease is a periodic one, appearing in the spring and early summer and disappearing again as colder weather sets in. The symptoms are described by **Goldberger**[4] as follows:

> In a fairly well developed though not advanced case the disease shows itself by a variety of symptoms, of which weakness, nervousness, indigestion, and an eruption form the most distinctive combination. The eruption is the most characteristic telltale of the disease and the main reliance in its recognition. When the eruption first shows itself it may look very much like a sunburn, the deceptive resemblance to which may, in some cases, be heightened by the subsequent peeling with or without the formation of blisters. In many cases the inflamed-looking skin first turns to a somewhat dirty brown, frequently parchment-like, then quickly becomes rough and scaly, or cracks and peels. In many instances, however, the beginning redness is not noticed or does not occur, the first and perhaps the only thing observed being the dirty-looking scaly patch of skin very much like and frequently thought to be no more than a simple weathering or chapping.
>
> Among the most distinctive peculiarities of the eruption is its preference for certain parts of the body surface. The backs of the hands in adults and the backs of the feet in children are its favorite sites. Other parts not infrequently attacked are the sides or front of the neck or both, the face, elbows, and knees.

[2] **Siler**, Proc. 2nd Pan. Am. Sci. Cong. Sect. 8, Pt. 2. p. 14.
[3] **Siler**, *Ib.*
[4] **Goldberger**, Pub. Health Repts. 33, 481, 1918.

From these or other points, for it may attack any part of the body, it may spread to a varying degree. Another marked peculiarity of the eruption is its tendency to appear at about the same time, and to cover similar areas, both as to extent and peculiarities of outline, on both sides of the body. Thus it may be stated as the rule that if the back of one hand or of one foot, one elbow, one knee, one side of the neck, one cheek, or the lid of one eye is affected, then the corresponding part on the other side of the body is affected, and affected to almost exactly the same extent. This rule, however, is not without many exceptions. It must not be assumed, because the back of one hand or of one foot or of one side of the neck alone seems to be involved, or is involved to so slight an extent as to be almost nothing in comparison with the involvement of the other side, that the possibility of the disease being pellagra may be thrown out of court without further ado.

Although the main reliance in the recognition of the disease, the eruption of pellagra, not infrequently is very tardy in making its appearance, while, until it appears, it is ordinarily impossible to determine the presence or absence of the disease with certainty, a shrewd suspicion may, nevertheless, be formed from a careful consideration of the other symptoms. This applies only to a limited extent to children, in most of whom the manifestations of the disease, other than the eruption, are slight and frequently difficult or impossible to make out. It has happened more than once that the liveliest of a group of children was found with a well-developed eruption. Notwithstanding this, however, careful questioning of the mother, if she be observant, not infrequently develops the fact that the child seems to her less active than common; in some cases it is evidently listless and fretful and the mother may also recognize that it has fallen off in weight. In older individuals a complaint of loss of strength with indigestion or nervousness, or both, coming on or made worse in the spring or summer and improving in the fall and winter, are very frequently met with. The patient will complain of being "worked out," of having "blind staggers" (dizziness, vertigo), of discomfort or pain in the pit of the stomach, frequently of headache, sometimes of wakefulness, frequently also of sluggishness of the bowels, requiring the habitual use of medicine to move them. Although, as has already been said, these symptoms alone or even with the addition of such symptoms as a burning or scalded feeling of the mouth, reddened tongue, burning of the hands or feet and loose bowels are not enough to distinguish pellagra from other conditions, they are ample to justify a suspicion of the disease, especially if such individual is known to be finicky or a nibbler about food, or has been living on a diet made up largely of biscuits, corn bread, grits, gravy, and sirup, with little or no milk or lean meat; in other words, on a diet mainly of cereals, starches, and fat, with but little, if any, of the animal flesh (protein) foods.

As the disease progresses the patient loses strength and flesh, the skin shrivels and the bones become prominent. The muscles waste and the movements are slow and languid. The nervous symptoms consist first in a melancholia, later the delirium may become maniacal in character; twitchings, tremors or epileptic seizures are frequently seen.

Boyd,[5] discussing the disease as it appeared among the Turkish prisoners in Egypt, states that the first symptoms appeared to be

[5] **Boyd**, Roy. Soc. Med. Sec. Ther. and Parm. Nov. 1919, Lanc. 1919, ii, 979.

loss of appetite, followed by failure of hydrochloric acid, failure of pancreatic juice, failure to assimilate, decomposition of protein, and finally excretion of indican. There was also severe loss by diarrhoea. Metabolic observations were made upon Turks who did not show these symptoms, and it was found that their excretion of fat and protein were both well above the normal, showing that they were already in what might be called a pre-pellagrous state.[5a]

Lewis [6] reports that the chemical composition of the blood of pellagrins shows no abnormal deviations, but degenerative changes have been observed in the tissue [7] and in the cells of the sympathetic nervous system.[8]

According to **Nicolaidi,**[9] a comparison of analyses of feces, urine, and food of pellagra patients shows that considerable demineralization occurs in the organism; there is a pronounced decrease in the amount of nitrogen, phosphates, magnesia, lime, and potash excreted in the urine and a surprising increase in the amount eliminated in the feces. Comparative experiments with patients suffering from other similar affections showed that this derangement of absorption is quite characteristic for pellagra. **Hunter, Givens** and **Lewis** [10] found, however, that pellagrins have no difficulty in maintaining nitrogen balance even on moderate amounts of protein in the food.

Sullivan, Stanton, and **Dawson** [11] state that the mineral metab-

[5a] Various theories regarding the cause of pellagra are reviewed by **Viswalingam** (J. Trop. Med. Hyg. 21, 153, 1918; 23, 46, 1920; Expt. Sta. Record 43, 263). Further evidence is given in confirmation of the view that both the deficiency and the parasite theory hold good in the etiology of the disease among the Chinese in the Malay States. The freedom of the Malay and Tamil laborers from the disease is thought to be due chiefly to the better quality of their diet, which includes a variety of fresh vegetables and more or less meat. That faulty diet is not solely responsible for the disease is shown by the seasonal recurrences of symptoms in patients placed in a hospital with adequate diets for considerable periods of time. "Whether the infecting agent is an organism which enters the gastro-intestinal system and produces a toxin which gets absorbed into the system and produces the varied symptomatology, or whether owing to a deficiency in the vitamins some deleterious products are created in the intestines and give rise to an intoxication of the system, it is difficult to say at present."

[6] **Lewis,** Hyg. Lab. Bull. 116, p. 37, 1920.

[7] **Sundwall,** U. S. Pub. Health Bull. 106, 5, 1917.

[8] **Roof,** Roy. Soc. Med. Sect. Ther. and Pharm. Nov. 1919; Lanc. 1919, ii, 979. See also **Vedder,** 3rd Rep. **Thompson,** Pell. Com. p. 340; **Roberts,** Pellagra, St. Louis, 1912; **Wood,** Treatise on Pellagra, New York, 1912; **Pollock** and **Singer,** Arch. Int. Med. 11, 565, 1913.

[9] **Nicolaidi,** Zentr. Bioch. Biophys. 15, 815; 16, 676.

[10] **Hunter, Givens,** and **Lewis,** Hyg. Lab. Bull. 102.

[11] **Sullivan, Stanton,** and **Dawson,** Arch. Int. Med. 27, 387, 1921.

olism of pellagrins seems to be abnormal, especially in the actively pellagrous state, as instanced by the low phosphorus excretion, despite the fact that the diet taken at the time of the observations was generally one with abundance of milk. There were indications of heightened putrefaction in the intestines, and casts or albumen or both in the urine gave evidence of more or less kidney change in about 50 per cent of the cases, although marked pellagra can occur with no evidence of kidney change. They observed low excretion of total nitrogen and ordinary urinary constituents. The urea ration in general was low, and in certain cases with fair total nitrogen the urea ratio was lower than should be expected, a finding which suggests liver insufficiency. The low excretion of uric acid and creatinin during the active stages of the disease shows a low level of metabolism. The utilization of protein was found to be subnormal, even after several weeks of remedial diet.

Murlin[12] found that the quantity of hippuric acid excreted by pellagrins, especially those kept on a corn-vegetable diet, was from two to three times the quantity excreted by normal men on a normal diet, and suggests that this may be a factor of some importance in connection with the disease.

Voegtlin and Harries[12a] made chemical analyses of the breast milk from five cases of uncomplicated pellagra and found no striking deviations in composition from normal human milk. Lactose, fat, total N, and total solids were within normal limits but below the average. Casein and lactalbumin showed but a very moderate diminution. Total ash and P_2O_5 content were normal. Cl and Na were present in larger amounts than normal. The percentage of Ca, Mg and K was slightly below normal. Tests were not made for the vitamin content of the milk, and it may possibly have been lacking in these compounds. The total volume of the milk was frequently greatly diminished. Both total N and non-protein N in the milk were apparently increased by a change from a low-protein to a high-protein diet.

Gastric digestion is frequently below normal, due to hypoacidity[13] and to reduced pepsin content[14] in the gastric juice. **Preti and Pollini**[15] report that several enzymatic processes are either entirely suppressed or proceed irregularly. In many cases neither rennin nor rennin zymogen could be detected and failure of the preteolytic and amylolytic power of the feces was also observed. Some cases are characterized by profuse diarrhoea, others by persistent constipation.

[12] **Murlin**, Hyg. Lab. Bull. 116, p. 45, 1920.

[12a] **Voegtlin and Harries**, U. S. Public Health Ser. Hyg. Lab. Bull. 116, 73, 1920.

[13] See **Meyers and Fine**, Am. J. Med. Sci. 145, 705, 1913.

[14] **Hunter, Givens, and Lewis**, Hyg. Lab. Bull. 102.

[15] **Preti and Pollini**, Rif. Med. 1911, No. 27; Zentr. Bioch. Biophys. 13, 54.

A great variety of theories have been advanced as to the cause of pellagra. From the frequency of its occurrence among maize-eating people it was at one time believed that this grain or some constituent of it was responsible,[16] but this hypothesis, so far at least as it supposes a toxic substance in or formed from maize, has been discredited.[17]

Voegtlin [18] concluded from the clinical and pathological aspects of the disease that it represents a chronic intoxication from some unknown substance in the vegetable food, but notes that deficiency of either vitamins or amino acids might also be accountable.

Raubitschek [19] regarded the symptoms as due to a photodynamic action of sunlight in the presence of some constituent of corn. This view is supported to some extent by **Suarez**,[20] but disputed by **Ruhl** [21] and **Rondoni**.[22] **Allesandrini** and **Scola** [23] suggest that pellagra is silica poisoning, due to the presence of colloidal silica in the drinking water. **Aulde** [24] has advanced the theory that calcium depreciation is responsible. **Blosser** [25] ascribes it to excess of sugar cane products in the diet, since exclusion of partially refined sugars and sirups from the diet was followed by a cure in 121 of his cases and improvement in eight, while four died. **Yarborough** [26] regards it as an auto-intoxication (acidosis) due to a diet rich in carbohydrates. Certain authors have endeavored to trace a connection between the intestinal bacteria and pellagra.[27] Others [28] have regarded it as an infection, probably of a protozoal nature, carried by sand flies. All attempts

[16] See **Horbaczevski**, Gas. Lek. Ces., 1920, No. 37; Zentr. Bioch. Biophys. 10, 932; **Carbone** and **Cazzamalli**, Giorn. r. soc. ital. d' igiene, 1914, 5, 51, 99, 151; Zentr. Bioch. Biophys. 17, 635, 1915; **Volpio**, Pathologica, 5, 174; Zentr. Bioch. Biophys. 16, 370; **Volpino** and **Bordoni**, Pathologica, 5, 602; Zentr. Bioch. Biophys. 18, 459, 1915; **Rondoni**, Sperimentale, 69, 723, 1915; Trop. Dis. Bull. 7, 63, 1916.

[17] **Sambon**, Brit. Med. J. 1913, ii, 1, 9, 297.

[18] **Voegtlin**, J. Am. Med. Ass. 63, 1094, 1914.

[19] **Raubitschek**, Centr. Bakt. 1, Abt. Orgi. 57, 193; Wiener Klin. Wochens., 1910, 963.

[20] **Suarez**, Bioch. Z. 77, 17, 1916.

[21] **Ruhl**, Dermatol. Woch. 60, 113, 151, 176, 1915; Trop. Dis. Bull. 7, 65, 1916.

[22] **Rondoni**, Lo speriment, 65, 307; Zentr. Bioch. Biophys. 12, 716.

[23] **Allesandrini** and **Scola**, Z. Chemother. Orig. 2, 156.

[24] **Aulde**, J. Med. Rec. 90, 181, 1916.

[25] **Blosser**, South Med. J. 8, No. 1; J. Am. Med. Ass. 64, 573, 1915.

[26] **Yarborough**, Med. Rec. Sept. 1916.

[27] **Neusser**, Wien. Med. Presse, 1887, iv, 146. De. Giaxa, Annali d'Igien. Soer. Roma, 1892, 1903; **McNeal**, Am. J. Med. Sci. 145, 801, 1913.

[28] **Sambon**, J. Trop. Med. 13, 271, 287, 305, 319, 1910; **Roberts**, J. Am. Med. Ass. 56, 1713, 1911.

to transmit the disease from one person or one animal to another have failed, however.[29]

The Thompson Pellagra Commission[30] has compiled a mass of evidence which they believe indicates that the disease is communicable and has its origin in defective sanitary conditions. They state that new cases usually occur during or within six months of residence in close proximity to a pellagrin in the active stage of the disease, and that the installation of a water-carriage system of sewage disposal at Spartan Mills, without any change in the habits or mode of life of the inhabitants, transformed the community from a pellagra focus to one in which pellagra no longer spreads. Although old cases still suffer recurrence, even these have decreased in number.

Jobling and **Peterson,**[31] in their survey of pellagra in Nashville, came to practically the same conclusions as the Thompson Commission. Their observation that a close relationship exists between the sanitary condition of the different parts of Nashville and the incidence of pellagra, tends to strongly support the view that the disease is associated with poor sewage disposal. The sanitary conditions in those districts where pellagra is common are of the worst sort, in many instances there being little pretense made of doing anything with the excreta, which in summer is covered with flies. Screening was usually absent from those houses where the disease was found. **Vedder,** on the other hand, holds that the proofs offered in support of this theory are insufficient and susceptible of other interpretations.[32] He says:

> To accept this zone distribution of cases as pointing to an infectious agent as the cause of pellagra appears to involve an inconsistency. We must necessarily assume that the infectious agent is conveyed in some manner from house to house, and also from the frequency with which contiguous houses are attacked that it is susceptible of being distributed with a considerable degree of certainty. In other words, we must assume that the organism causing the disease must be very highly infectious if it is capable of making it dangerous to live next door to a case of pellagra. Yet, in institutions, pellagra in a doctor, nurse or attendant is so rare as to be a curiosity. Here there is an infection so powerful that it often spreads from house to house, and yet which is powerless to attack attendants performing the most intimate of personal services. Such a peculiar incidence is conceivable in a disease carried by an insect, if the hospital attendants were protected from that insect. But as the method of transmission is unknown, measures to prevent insect transmission could not have been intelligently adopted in these institutions, many of which are infested with bedbugs, lice, biting flies and mosquitoes.

[29] **Lavinder, Francis, Grim,** and **Lorenz,** J. Am. Med. Ass. 62, 1093, 1914; **Goldberger,** Publ. Health Repts. 31, 3159, 1916.

[30] **Siler, Garrison** and **McNeal,** 2nd and 3rd Repts. Thompson Pellagra Commission.

[31] **Jobling** and **Peterson,** J. Infect. Dis. 18, 501, 1916.

[32] **Vedder,** 3rd Rep. Thompson Pell. Com.

Is there a tendency of pellagra to occur in that part of a community having a primitive system of disposal of excreta and to be absent in the portion having a proper sewer system? The commission has shown that pellagra in Spartanburg is much commoner in those sections of the city having privies than in those sections of the city having a sewer system. The sewer system runs throughout the business sections and better residence districts, while the poorer residence districts, including the several foci of mill workers, are not thus supplied. . . . The commission points to the epidemic of pellagra occurring in Peoria as an instance of the occurrence of the disease in a well-sewered institution, but thinks that the disease in this case may have been spread by contact. While freely granting this, we must see that the occurrence of such an extensive epidemic of pellagra in a well-sewered institution must necessarily detract from the importance of the sewer system alone as a factor in reducing the prevalence of pellagra in the sections of Spartanburg it supplies. It should further be remembered that as pellagra in the Peoria institution disappeared after a radical change for the better had been made in the dietary, together with the enforcement of segregation of pellagrins, the disappearance of the disease cannot logically be attributed to the latter fact alone. Of course a brief discussion of one or two isolated instances can not do justice to the large number of observations accumulated by the commission on this point, but apparently the commission does not believe that they have any direct proof that pellagra has disappeared as the result of improved methods of conservancy alone.

It is to be noted in this connection that **Boyd** states that the sanitation and water supply was entirely satisfactory in the prison camps in Egypt in which the disease developed, and that an exhaustive search for any causative origin other than a faulty diet was without result.

Goldberger is the leading exponent of the theory of dietary origin for pellagra.[33] Investigation has shown that the diet of pellagrins is almost invariably restricted and ill-balanced. Fat and carbohydrate predominate, and animal protein is low, as may be seen from the following diets taken from those reported by **Vedder**[34] as typical:

Miss T., mill operative. Breakfast. Hot biscuit, butter and molasses; rice, occasionally; canned salmon frequently; eggs, a couple of times a week; coffee, glass of milk at times.

Dinner: Meat, usually bacon boiled with vegetables; had fresh beef once or twice a week, fried and usually overcooked; chicken or rabbit about once a month; had vegetables, such as Irish potatoes, sweet potatoes; in winter used canned vegetables, but in summer had string beans, corn, peas, onions, etc., cornbread made of shipped meal.

Supper: Cornbread and buttermilk; drank about a quart of milk daily (her estimate); ate a good deal of candy and always had plenty of fresh fruit.

[33] **Goldberger**, Pub. Health Repts. 20, 1683, 1914; *Ib.*, 30, 3117, 3338, 1915. **Goldberger** and **Wheeler**, *Ib.*, 30, 3, 1915. See also, **Vedder**, Arch. Int. Med. 18, 137, 1916. **Goldberger, Wheeler** and **Sydenstricker**, J. Am. Med. Ass. 71, 944, 1918. [34] **Vedder**, 3rd Rep. Thompson Pell. Com.

This family (father and mother and seven children from 20 years of age to 13 months) purchased each month: 100 pounds of wheat flour, two bushels corn meal, one bushel Irish potatoes, hominy and rice as desired from time to time, and no account kept.

Mrs. H., housewife in mill village: Breakfast: Biscuits or bread; salmon twice a week, other mornings bacon; coffee, eggs rarely.

Dinner: Tomato soup; salt pork boiled with vegetables, usually cabbage, sometimes string beans; Irish potatoes; fresh meat only on rare occasions in the winter, never in summer; biscuit or corn bread.

Supper: Bread with milk when they can get milk; bread is the main part of supper with anything left over from dinner; occasionally something canned. There are nine in the family, with seven children, from fourteen years to four months, and they purchase every month: 100 pounds flour, one bushel corn meal, one peck Irish potatoes.

Mrs. B., wife of mill operative. Breakfast: Rice, oatmeal occasionally, hominy frequently, bread, butter and jelly; coffee; eggs several days a week; in winter fresh meat once a week.

Dinner: Irish potatoes, bacon and vegetables, beans, peas, or turnip greens; bread and butter.

Supper: Irish potatoes, cornbread sometimes, otherwise wheat bread, butter, and anything left from dinner; drinks no milk; has a chicken occasionally, but not often.

Vedder sums up his observations as follows:

In general it may be said that the great bulk of the food of these poorer pellagrins consists of wheat flour, cornmeal, potatoes, salt pork and boiled vegetables, and that during the winter even these latter are scarce and consist chiefly of beans and cabbage. In some instances a considerable amount of canned meats and vegetables were used, but most of the pellagrins did not use these to any extent. It will be seen that the great bulk of the food consisted of carbohydrates and that protein foods such as meat, milk and eggs are relatively little used. It is also important to notice that wheat flour, cornmeal and salt meat are deficient in both scurvy and beriberi vitamins. Potatoes possess the scurvy vitamin, but are relatively very low in beriberi vitamins. It has been demonstrated that the vitamins present in canned goods may be destroyed by the sterilization to which they are subjected. It is therefore clear that these people are living in great part on foodstuffs the continued and disproportionate use of which will produce either beriberi or scurvy, or both. It may be asked why these people do not suffer from these diseases. This is because they all eat sufficient fruit or fresh vegetables to protect them from scurvy, while many of them eat peas and beans frequently. These rank as good preventives of beriberi. But it is quite reasonable to suppose that possibly there is some third deficiency in wheat flour, cornbread, etc., from which they are not protected by these additions. Generally

speaking, and with the exception of meat, which is not used largely by the poor, the diet is much more limited in the winter. If pellagra is a deficiency disease, it is during these winter months that it develops and the symptoms appear in the spring. At about that time eggs, milk, fresh vegetables and fruits become abundant and cheap, are used in considerable quantities as compared with the winter diet, and the patients for the most part recover, only to develop another recurrence the following spring after another winter on a comparatively limited diet.

To the cases above mentioned, all of which came from the poorer classes, whose diet is largely governed by necessity, may be added two or three coming from well-to-do families:

Mrs. D., housewife in comfortable circumstances. Breakfast: Grits or oatmeal, ham or bacon; eats an egg about two mornings a week; bread, biscuit and postum. States that the chief part of her breakfast is bread and butter and postum.

Dinner: Soup (canned), beans, and Irish potatoes; cabbage, corn and other vegetables in summer, but all winter main part of dinner is beans, and potatoes; bread and often pie or cake; one glass buttermilk.

Supper: Same as dinner.

Meat is served often but patient states that she does not care for it and almost never eats it. Three adults in family purchase monthly: 100 pounds flour, one peck cornmeal, one peck potatoes.

Mrs. B., wife of hardware dealer in comfortable circumstances. Breakfast: Hominy and other cereals; one egg, nearly every morning; biscuits or toast or batter cakes with coffee; very rarely a small piece of steak.

Dinner: Macaroni, occasionally, usually Irish potatoes or rice, steak is served for the rest of the family, but she never eats it. States that she never has cared for meat. Occasionally had chicken or fish and might eat a little of these. Practically no vegetables in the winter, when she ate almost nothing but potatoes and bread. Even during the summer, when vegetables were plenty, she ate mostly potatoes, as she preferred them. Has also been a hearty bread eater, and eats more of this than anything else. Says she could almost live on bread.

Supper. Just bread and jelly. Has eaten this for supper as long as she can remember. Did not drink milk, and used very little butter. Has continued cutting one thing after another out of her diet because she thought the foods thus omitted were the cause of indigestion, and for nearly a year before her illness, lived chiefly on bread and potatoes.

Mrs. T., housewife in well-to-do circumstances. Breakfast: Either oatmeal or grits; bacon or ham; usually ate an egg several times a week; bread, butter and coffee.

Dinner: Usually salt pork boiled with vegetables. During the winter when there were not many vegetables, used canned goods. Also had canned salmon and salt fish. She served fresh beef several times a week, but she did not care for it and seldom ate it. Ate a small piece of chicken several times a week. She always had boiled rice and also Irish potatoes and ate freely of both, and also of bread. Usually had cake and stewed fruit for dessert.

Supper: Hominy with either cheese, ham or bacon, and rarely eggs. She ate a fair amount of hominy and a considerable amount of bread. States that she is a little inclined to be a vegetarian because she does not like to have animals killed. Drank no milk, ate very few eggs, and bought a large amount of canned goods.

Mr. S., a freight conductor. A very large and muscular man weighing 245 pounds and apparently a very well-nourished man. Eruption of pellagra pronounced, but no other symptoms except that he lost fifteen pounds weight just prior to the appearance of the erythema.

Breakfast: Gets a very early start and usually eats no breakfast. When he does it consists of coffee and a biscuit.

Dinner: Almost always eats Irish potatoes fried with onions, and eats more of these than anything else; cabbage often. Does not eat meat because he does not care for it. Says he does not eat a pound of meat in six months. Has eggs perhaps once a week; beans occasionally; bread; ice cream often.

Supper: Potatoes and onions again whenever he can get them; oysters or fish occasionally, otherwise sandwiches or pie.

Stated that he might drink a glass of milk a day, and never ate butter, but that he was very fond of fresh raw eggs, and when he found some fresh ones, he might eat half a dozen at one meal. Said this did not happen more than once or at most twice a month. His dietary habits were most erratic, as he had to pick up what he could get on the road a good deal of the time, but he almost always ordered potatoes and onions for which he had an especial predilection.

Vedder says that in practically every case there is some peculiarity of taste or a history of indigestion or some other circumstance, as a result of which the patient had lived on a very one-sided diet, in every case consisting chiefly of flour, or corn products or potatoes, often with the addition of salt pork in some form or canned vegetables. Similarly **Roberts** [35] describes 25 cases from private practice, all of whom were amply able to provide an adequate diet for themselves, but all of whom for one reason or another ate unbalanced or insufficient food.

Goldberger experimented with two orphanages at Jackson, Miss., which had been pellagra centres for several years. Without altering the hygienic and sanitary conditions the diet in the two institutions was modified to as to include a much larger proportion of fresh animal and leguminous protein. Among the 340 inmates from the two institutions who were kept under observation for a year only one developed a recurrent attack and no new cases were seen. Precisely similar results were obtained at the Georgia State Sanitarium, where a modification of the diet on the same lines as those followed in the orphanages had equally good effect, no recurrence being observed among the 72 pellagrins under observation, whereas among the control cases kept on the accustomed diet, 47 per cent presented recurrences.[36]

[35] **Roberts,** J. Am. Med. Ass. 75, 21, 1920.

[36] **Goldberger, Waring,** and **Willets,** Pub. Health Repts. 30, Oct. 22, 1915.

A very interesting experiment was carried out at the farm of the Mississippi State Penitentiary, where eleven convicts were induced by the offer of a free pardon from the Governor to submit themselves to an experimental diet similar to that on which pellagra was known to develop. The remaining population of the farm were kept under observation as controls. The volunteers kept about the same hours and did about the same kind and the same amount of work as the other convicts. Such differences as existed were in favor of the volunteers, especially during the latter portion of the experimental period. The general sanitary environment was the same for volunteers and controls, but the hygienic environment — personal cleanliness, cleanliness of quarters, freedom from insects, particularly bedbugs — was decidedly in favor of the volunteers. The diet comprised patent wheat flour, corn meal, corn grits, corn starch, white polished rice, standard granulated sugar, cane syrup, sweet potatoes, pork fat (fried out of salt pork), cabbage, collards, turnip greens, and coffee. By the end of five months on this diet six out of eleven volunteers had developed symptoms justifying a diagnosis of pellagra, including the typical eruption, nervous and gastro-intestinal symptoms.[37] This seems to be well attested, although **McNeal**[38] has denied that the observed symptoms were those of pellagra.

Three investigations have been reported in which symptoms closely resembling those of pellagra have been produced in animals by prolonged feeding on unsuitable rations. **Chittenden and Underhill**[39] fed dogs on a diet consisting of varying quantities of boiled peas, cracker meal and cottonseed oil or lard. The animals were kept under the best sanitary conditions, protected from any possible infection, and allowed a sufficient amount of exercise, so that the only abnormal condition was the diet. After periods varying from one to eight months a pathological condition strikingly similar to pellagra made its appearance. At times the animals could be restored to normal condition by the addition of meat to the diet. The same symptoms could be induced on a diet of meat, cracker meal and lard, provided the meat intake was sufficiently small, but less than 50 per cent of the dogs were susceptible to these conditions. The investigators state that the level of nitrogen intake as such plays little or no rôle in the development of this disease.

Clementi[40] found that after a prolonged exclusive diet of maize,

[37] **Goldberger and Wheeler**, Pub. Health Repts. 30, Nov. 12, 1915.

[38] **McNeal**, J. Am. Med. Ass. 66, 975, 1916.

[39] **Chittenden and Underhill**, Am. J. Physiol. 44, 13, 1917.

[40] **Clementi**, Rass. Clin. terapi. sci. affini. 16, 121, 1917.

chickens develop disturbances of digestion and of nervous system very different from those produced by polished rice and analogous to those of pellagra in man or those produced in birds by injections of alcoholic extracts of rotten maize. On discontinuing this diet the symptoms slowly disappear.

Chick and **Hume** [41] state that monkeys fed on a low-protein diet made up of butter, banana or apple, sugar, orange juice, cornflour biscuit, and " marmite " (a commercial yeast preparation) lost weight slowly and gradually developed symptoms characteristic of pellagra.

The food deficiency theory receives further support from the beneficial results of a rich and varied diet in the treatment of the disease. Even those authorities who disagree with this theory as to the origin of the disease concur in advocating an improved dietary as a means of combating its development. **Siler** [42] states that his advice is always to build up the nutrition particularly by the use of milk and eggs, and that in a large majority of cases, though not all, the results are good.

Goldberger [43] asserts that " For the cure of pellagra the only medicine we have is the diet." For both prevention and treatment, the diet should be well balanced, including a sufficient amount of milk or lean meat (fowl, fish, pork, beef, etc.) and an abundance of green vegetables (cabbage, collards, turnip greens, spinach, string beans, snap beans) and fruits (apples, peaches, prunes, apricots). At least 1.5 pints of sweet milk or buttermilk should be taken daily. If but little milk be taken, 0.5 pound of lean meat should be eaten at least three or four times a week. Eggs or cheese may partly replace, or better still, be eaten in addition to the meat. Dried soy bean is highly recommended as a substitute for meat. It may be eaten either boiled or baked. A portion of the flour or corn meal used in biscuit or corn bread may be replaced by soy bean meal.[43a]

Ridlon [44] used the following diet in the treatment of pellagra:

Patients received one quart of fresh milk a day, two eggs, poached or soft boiled, a day; wheat bread three times a day, butter, one-half ounce, three times a day; coffee once a day if desired; fresh, lean meat

[41] **Chick and Hume,** Bioch. J. 14, 135, 1920.

[42] **Siler,** Proc. 2nd Pan. Am. Sci. Cong. Sect. 8, Pt. 2, p. 12.

[43] **Goldberger,** Pub. Health Repts. 33, 481, 1918.

[43a] Symptoms resembling pellagra in the human being have been produced on a diet lacking in antineuritic principles and consisting of protein 40 g., carbohydrates 462 g., fat 109 g., ash 30 g., per day. The calorific value therefore would be about 3070 calories. (**Sullivan** and **Jones,** Hyg. Lab. Bull. 120, 117, 1920. See also Sullivan, *Ib.* p. 127.)

[44] **Ridlon,** Pub. Health Repts. 31, 1979, 1916.

three times a day, either beef, mutton, chicken, veal, or pork; oatmeal or wheat once a day; dried beans or peas either baked or boiled or made into a soup, once a day; one other vegetable, either fresh legumes, turnips, carrots, onions, potatoes, or cabbage, once a day; stewed fruit three times a week; and rice once a week. In addition the patients were allowed fruit juices or fresh fruit, as apples, oranges, or peaches, in season.

This diet includes a large proportion of the animal and vegetable proteins and there is also a diminution in the proportion of sugars and starches.

No sirup, corn products, or fat white meat (salt fat pork), and no canned vegetables were allowed, not because these articles in themselves are unwholesome, but because experience has shown that, alone or in combinations, they have commonly formed a conspicuously large portion of the prepellagrous diet.

Two groups were compared, those receiving only the dietetic treatment outlined, and those receiving medication as well. **Ridlon** states that the thirteen patients receiving no medication were comparable as to severity and in all other respects to the eighteen patients who received various drugs in addition to the dietetic treatment. The latter group made more rapid or marked improvement than the former. The average duration of stay under treatment of the fifteen patients receiving medication who improved was 85.8 days, and the average gain in weight 11.7 pounds. The shortest period under treatment was 26 days, and the longest 189 days. The average duration of stay under treatment in the thirteen patients receiving only dietetic treatment was 83.5 days, and the average gain in weight was 18.3 pounds. The shortest period under treatment was 29 days, and the longest 191 days. Patients showing predominating symptoms of the skin and alimentary tract as a rule show a ready response to treatment, and improvement is noted within a week to ten days. On the other hand, those who have suffered from repeated attacks and have sustained a serious danger to the nervous system require a longer period for the repair of this damage.

Ridlon concludes that in pellagra the dietetic treatment is of paramount importance, the successful diet being one in which the animal and leguminous protein component has been relatively increased and the non-leguminous vegetable component relatively decreased.

Roberts[45] recommends feeding on at least 2,000 calories per day and as much more as can be given with safety, with special emphasis on lean meats, eggs, milk, butter, and vegetables containing proteins.

[45] Roberts, J. Am. Med. Ass. 75, 21, 1920.

Experience in other countries runs parallel with that of American physicians. **Bravetta**[46] asserts that good food represents the best remedy to assuage, to cure and perhaps to cause the complete disappearance of pellagra.[47]

If we allow that there is strong presumptive evidence for the view that pellagra is caused by deficiency in the diet we are at once confronted with the problem of determining the nature of the deficiency.

Funk[48] classed pellagra along with scurvy as due to lack of vitamins, and later observers have for the most part held this as at least a possible contributary factor.[49] **Voegtlin, Neill and Hunter**[50] have conducted an interesting study on the effect of adding vitamin preparations to the ordinary diet of pellagrins in amounts based on the body weight of the patients and calculated from the doses necessary to cure severe polyneuritis in fowls. The vitamin preparations used were of two different types, those from animal sources (liver and thymus gland) and those from vegetable sources (**Funk's** vitamin fraction from yeast and rice polishings). The liver and thymus extracts were tested and found to possess antineuritic properties, though they were less effective in this respect than the yeast or rice preparations. The liver extract, at least, and possibly the thymus extract as well, must have contained vitamin *A* as well as *B*.[51] Although the results are not entirely conclusive they appear to indicate that the administration to cases of pellagra of these preparations made from yeast and rice polishings over a considerable period of time and in large amounts (based on the curative dose for fowls) in general failed to modify the course of the disease.

The administration of the liver and thymus preparations to pellagrins was followed by an improvement in their condition apparently comparable to that produced by the consumption of a diet rich in fresh animal proteins.

The investigators conclude that the evidence presented clearly indi-

[46] **Bravetta,** Riv. pellagrolog. ital. 15, 43, 1915. Tropical dis. Bull. 7, 59, 1916.

[47] See also **Rondoni,** Sperimentale, 69, 723, 1915. Trop. Dis. Bull. 7, 63, 1916.

[48] **Funk,** Munch. Med. Wochenschr. 61, 698, 1914.

[49] **Goldberger, Wheeler, and Sydenstricker,** Pub. Health Repts. 35, 648, 1920; **Chittenden and Underhill,** Am. J. Physiol. 44, 13, 1917; **Voegtlin and Harrie,** Hyg. Lab. Bull. 116, 84, 1920; **Vedder,** 3rd Rep. Thompson Pell. Com. 338-372; **Voegtlin, Sullivan and Myers,** Publ. Health Repts. 31, 935, 1916; **Wood,** Sci. Am. Supp. 82, 43, 1916.

[50] **Voegtlin, Neill and Hunter,** Hyg. Lab. Bull. 116, 1920.

[51] **Osborne and Mendel,** J. Biol. Chem. 34, 17, 1918.

cates that the dietary defect responsible for pellagra is distinctly (qualitatively) different from and perhaps more complex than the one causing fowl polyneuritis and human beriberi, a conclusion in accord with that reached by **Clementi** from his observations on fowls.[52]

It might be seen that the experiment of **Chick** and **Hume** referred to above (p. 254), in which the symptoms of pellagra were produced in monkeys, would eliminate the possibility of any connection between the known vitamins and this disease, since their ration included butter-fat as a source of *A*, yeast as a source of *B*, and orange juice as anti-scorbutic. The ration used was, however, like all pellagra-producing diets, ill balanced, being characterized by low protein and excessively high carbohydrate and fat. According to **Funk** and **Dubin**,[53] such a diet requires the addition of extra vitamin *(B)* in order to maintain health. It follows, therefore, that vitamin deficiency is probably invariably a complicating factor in the disease.

McCollum has shown that the deficiency in **Chittenden** and **Underhill's** experimental diet was *A*, mineral salts, and poor quality of protein, since his experiments have demonstrated that all the important seeds can be rendered dietetically complete by addition of these factors.[54] The same deficiencies are commonly observed in the diet of pellagrins. McCollum believes, however, that pellagra is caused by an infectious agent to which individuals may be rendered susceptible through lowered vitality due to faulty diet. The experiments with rats are said to indicate that " health and vigor are promoted by a liberal intake of protein of good quality better than by any diet in which there is a tendency towards parsimony with respect to this dietary factor."

All recent work tends to emphasize the quality rather than the quantity of protein used. **Boyd**, in the report already referred to on pellagra among the Turkish prisoners in Eygpt, states that: Examination of the diet showed no connection between incidence of pellagra and either total calorific value or fat content or total protein content of the diets. It was only when the conception of the biological value of the protein was applied that any light was thrown upon the connection between diet and incidence of pellagra. The human body must have a certain amount of specific amino acids, and the different proteins have different biological values according to their power to supply these essential building materials, and so maintain the body

[52] **Clementi, Rass. Clin. terapi. Sci. Affini. 16, 121, 1917.**

[53] **Funk and Dubin, Sci. 52, 447, 1920.**

[54] **McCollum and Simmonds, J. Biol. Chem. 32, 29, 181, 347, 1917; *Ib.*, 33, 303, 1918. McCollum, Simmonds, and Parsons, *Ib.*, 33, 411, *Ib.*, 38, 113, 1919.**

in nitrogen equilibrium. If meat is regarded as having a biological value of 1.0, milk is found to have a value of 0.96, beans of 0.8, and corn (maize) of only 0.298. It is only when the biological value of the protein in a diet falls below 40 that pellagra begins to appear. Failure to absorb may raise this value as does also the performance of heavy work. For treatment, increase in the diet of protein of high biological value was found to cause the most rapid improvement, and administration of hydrochloric acid was helpful.[54a]

A similar conclusion was reached by **Wilson.**[55]

Observations have been made by **Wilson,**[56] upon the etiology of pellagra, and go to prove that the disease is to be attributed principally if not entirely to a defective protein supply in the diet. This conclusion emerged after a careful analysis of a large series of diet known to have produced pellagra, together with others, proved to be preventive or curative. Special attention was paid to the protein provided, not only as regards the actual amount, but also as regards the "biological value." The figures expressing the latter are taken from the work of **Thomas,** who investigated the nutritive properties of protein derived from various foodstuffs, and estimated their relative value in maintaining nitrogenous equilibrium in the human subject. These values were found to vary within wide limits, the "biological value" of vegetable protein (and especially of the protein derived from the corn grain) being less than that of milk or meat proteins. Equilibrium was not obtained with corn diet, but Thomas calculated from the data obtained that the amount of "tissue repair" effected by 30 grm. protein from meat or milk would require over 100 grm. of corn protein. Other foodstuffs, such as fish, rice, potatoes, occupied an intermediate position. The reason for this difference lies doubtless in the fact that animal proteins are more approximately constituted for animal nutrition as regards the amount and variety of their constituent amino-acids. The inferiority of corn proteins would be explained by the large proportion contained of zein, a protein which is devoid both of tryptophane and lysine, two amino-acids which are known to be essential for animal nutrition.

Unfortunately the results obtained from the different experiments with corn were not concordant, and further confirmation is necessary before Thomas's figure for the biological value of corn protein can be accepted as final. (See p. 264.)

It was after analysis of the pellagrous and non-pellagrous diets

[54a] During a pellagra outbreak in Egypt, **Bigland** (Lancet 1920, 1, 947) attempted to obtain organisms from the blood and feces on various media, complement fixation with alcoholic extracts of organs obtained at autopsy, inoculation of rabbits with pellagrous sera and the diagnostic use of maize extracts, but failed of positive results. An examination of the diets of these pellagrins showed that there was a deficiency in the "biological value of protein," but there were a few cases in which no such deficiency could be proved. To explain these cases, it is assumed that some toxin is formed which acts upon the intestine or its contents in such a way that protein is not properly assimilated.

[55] **Wilson,** quoted in Rep. 38, Med. Res. Com. London, p. 95, 1919.

[56] Unpublished work.

from this point of view that Wilson arrived at the conclusion that danger of pellagra occurred if the "biological value" of the protein ration was below a certain level. This level, as might be expected, varied within certain limits according to individual need and idiosyncrasy, hard manual labor needing a more generous provision in this respect. This view is in accord with many of the known facts of the incidence of the disease. For example, upon this theory pellagra would be likely to develop with greatest frequency upon a diet consisting largely of corn, owing to the low "biological value" of the proteins of this cereal; at the same time the disease might occur among wheat and rice eaters, in cases of an exceptionally low total consumption, but it could rarely occur among populations taking meat or milk even in small quantities. The protein deficiency which leads to pellagra, and is possibly concerned with a defective supply of some essential amino-acid or acids may, in Wilson's opinion, occur also upon an adequate diet if assimilation is defective as the result of gastro-intestinal disease. This theory would account for certain rare cases, occurring in England and elsewhere, of a disease with symptoms closely resembling those of pellagra. In these instances there has frequently been a history of chronic diarrhoea and gastro-intestinal disturbances of long standing.[57]

The observations of **Chick** and **Hume** on the effect of feeding specific proteins and amino acids to their pellagric monkeys is in accord with this view. The administration of tryptophane to one monkey staved off death for many weeks, while a mixture of lysine, arginine and histidine had little effect. In one case in which there were severe skin lesions the addition of five to 10 g. of casein produced a cure which is described as "dramatic."

While there is a growing tendency to regard pellagra as the result of a deficiency of certain essential amino acids it must be admitted that cases have been reported which are difficult to explain from this point of view. **Enright**, reporting on the outbreak of pellagra among the German prisoners in Egypt, states that:

"A careful analysis of the diets of these German prisoners before capture and during their period of captivity prior to the onset of the eruption, showed that they were ample, both in quantity and quality, for any possible requirements, and were of such varied composition that it is difficult to see how they could be improved."[58] He con-

[57] See also **McCollum** and **Simmonds**, J. Biol. Chem. 32, 350; 33, 303, 1917; **Roof**, Roy. Soc. Med. Sec. Ther. Pharm. Nov. 1919, Lanc. 1919, ii, 979.

[58] **Enright**, Lancet, 1920, i, 998. See also **Viswalingam**, J. Trop. Med. 23, 46, 1920.

cludes, therefore, that food deficiency cannot be regarded as the principal factor. **Goldberger**[58a] argues that the evidence offered by Enwright and also by Viswalingam is insufficient, inasmuch as the ration given may not have been consumed in adequate quantity, owing to racial differences in taste. Even more impressive are the cases reported by **Siler, Garrison,** and **McNeal,**[59] of pellagra appearing in tubercular patients who were on the rich diet of milk and eggs prescribed for that disease. On the other hand, as **Hess** has pointed out,[60] while adequate protein, milk, cheese, eggs, and meat, were all lacking to a pronounced degree in the Central Empires during the war, there was no marked prevalence of pellagra during these years.

It is obvious that more extensive investigations must still be made before we can entirely reconcile the conflicting evidence recorded. **Bory**[61] regards pellagra as a disease which can develop only under the conditions brought about by a one-sided diet, usually exclusively cereal, but believes that the infection is actually transmitted by some winged insect. This theory is supported by two cases recently encountered in France, one an Italian soldier who developed the disease in France after coming from a hot-bed of pellagra, and the other a sporadic case developing in a region in northern France where no other instance has been known before or since. A body of Italian troops had been camped in the vicinity for several weeks, and Bory believes that the mosquitoes which infested the district had become infected and carried the disease. The one man contracting it was predisposed, as he had lived for years on an exclusive diet of milk, rice, and other cereals on account of chronic digestive disturbance.

[58a] **Goldberger,** Lanc. 1920, II, 41.
[59] Third Rep. **Thompson** Pell. Com. p. 113.
[60] **Hess** and **Unger,** J. Am. Med. Ass. 74, 217, 1920.
[61] **Bory,** Prog. Med. 35, 461, 1920.

CHAPTER XV

MILK

THE constituents of milk are fat, carbohydrate, protein, salts, and vitamins, with 83 to 90 per cent of water. The carbohydrates, proteins, salts, and the vitamins *B* and *C* are in aqueous solution, while the fat is held in suspension as minute droplets which form an emulsion, in which the vitamin *A* is dissolved.

The milk of different species of animals varies considerably in the relative proportions of the constituents present; even within the same species, milk of different animals shows rather wide fluctuations round a mean. The following table[1] shows the general difference in the composition of human milk and cow's milk, from which it appears that human milk differs from cow's milk chiefly in its much higher sugar content and lower protein:

	Fat	Lactose	Protein
Human	2-4%	6-7.5%	0.7-1.5%
Cow	2-4%	3-5.5%	2.5-4%

Goat's milk has a higher content of protein than cow's milk, slightly lower sugar, and somewhat higher fat. Sheep's milk has a high content of both protein and fat.[2]

Fat is the most variable constituent in milk. Many factors influence the fat content of cow's milk, the breed, the individual characteristics of the animal, the period of lactation, and the season being the most influential. Sherman[3] gives the following figures as fairly representative of the breeds indicated:

Jersey	5.19 per cent fat
Guernsey	5.16 " " "
Durham	4.05 " " "
Ayrshire	3.64 " " "
Holstein	3.43 " " "

The fat content of human milk probably does not differ greatly

[1] Meigs and Marsh, J. Biol. Chem. 16, 167, 1913-14.

[2] See Folin, Denis and Minot, J. Biol. Chem. 37, 349, 1919, for analyses of milk of different species.

[3] Sherman, Chemistry of Food and Nutrition, N. Y., 2nd Ed. p. 59.

from that of average dairy milk, although the variability noted makes it difficult to generalize correctly. The results of a great many determinations gives an average of 3.9 per cent of fat in human milk. The composition of the milk fat varies considerably in different species,[4] owing to variations in the fatty acids present, but this appears to be of no importance in nutrition.

The lactose content of milk shows much less variation within the species than does the fat, but there is greater difference in this respect between different species, than is the case with the fat. **Mathews**[5] suggests that the high content of lactose in human milk may be correlated with the very much greater brain development of human beings. Myelin, a compound which develops rapidly in the brain fibres during the first six weeks of life, contains as one of its constituents galactose, which is produced by the hydrolysis of lactose. It may be, therefore, that one function of the lactose in human milk is to furnish this necessary substance for the development of the brain. If this is correct it is obvious that the deficiency of lactose in cow's milk cannot be satisfactorily remedied by the addition of cane sugar. In any case lactose is less susceptible to fermentation than is either glucose or cane sugar; consequently it is less liable to produce digestive disturbances than are these sugars.[6]

There is some uncertainty as to how many proteins are present in milk. In addition to the well-characterized caseinogen, and lactalbumin which make up by far the greatest proportion of the protein material, there is a third, lactoglobulin, which is more closely related to lactalbumin than to caseinogen[7] and also, according to **Osborne** and **Wakeman**,[8] an unidentified alcohol-soluble protein present in extremely small amounts. Besides these there are traces of non-protein nitrogen-containing substances, such as urea, creatine, creatinine and uric acid,[9] and possibly small quantities of proteoses (protein derivatives of lower molecular weight) as well.[10] The total proteins other than caseinogen are sometimes grouped together as " whey proteins," since they remain in solution in the whey when the casein is coagulated by acids or rennin.

[4] **Arnold**, Zeit. Unters. Nahr. u. Genussm. 23, 433, 1912.

[5] **Mathews**, Physiological Chemistry, 1915, p. 307.

[6] See also p. 267.

[7] **Lane-Claypon**, Oleomargarine, pp. 31–32, 38–41.

[8] **Osborne** and **Wakeman**, J. Biol. Chem. 33, 17, 1918.

[9] **Dennis** and **Minot**, J. Biol. Chem. 37, 353, 1919.

[10] Full details of the method of separation of the proteins of milk are given by **Osborne** and **Wakeman**, l. c.

Caseinogen is distinguished from the other milk proteins by its relatively high percentage of phosphorus and by the fact that it is coagulated by rennin. It is precipitated by heating in faintly acid solution, but redissolves in excess of acid or alkali. The action of the rennin enzyme is supposed to be a partial hydrolysis or digestion of the caseinogen, by which it is transformed into a new protein, casein, of lower molecular weight. The calcium salt of casein is insoluble and precipitates, forming a curd.

There is an unfortunate confusion in the nomenclature used by different authorities in connection with this phenomenon. While that given above is in accordance with English usage, the Germans give the name casein to the protein present in normal milk and distinguish the product of rennin action as paracasein. Many American writers follow the German usage, while others use the English nomenclature.

The clear fluid from which the coagulated casein has separated is known as whey, and contains the remaining proteins and other nitrogenous constituents as well as the milk sugar and inorganic salts.

Caseinogen probably exists in normal milk in the form of neutral calcium caseinogenate, Cas Ca_4.[11] The relation between this and the coagulum obtained by acidifying is not quite clear. It is completely precipitated from its neutral solution by saturating the solution with sodium chloride or magnesium sulphate or by half saturation with ammonium sulphate, or by the action of certain metallic salts such as alum, zinc sulphate, or copper sulphate.

Human milk contains a smaller percentage of caseinogen than cow's milk, although different investigators differ somewhat as to the relative proportions.[12] The caseinogen of human milk differs from that of cow's milk in being more difficult to precipitate by acid or to coagulate by rennin. Moreover, the curd formed from human milk is looser and more flocculent, and consequently more easily digested than the curd of cow's milk.[13]

Lactalbumin contains a higher proportion of sulphur and a smaller proportion of phosphorus than caseinogen. It is soluble in weak acid, but precipitated by addition of large quantities of mineral acids or metallic salts. At ordinary temperature it is soluble in half-saturated ammonium sulphate solution. It is not precipitated from neutral solution by saturation with sodium chloride or magnesium sulphate at ordinary temperatures, but separates on addition of acetic acid to this solution.

[11] **Van Slyke** and **Bosworth**, J. Biol. Chem. 20, 135, 1915.

[12] See **Lane-Claypon**, l. c. p. 34.

[13] **Hawk**, Pract. Physiol. Chem. 6th. Ed. p. 344.

Lactoglobulin has been less studied than caseinogen or lactalbumin. **Osborne** and **Wakeman**[14] suggest that it may be a lecithalbumin similar to the vitellin of hen's eggs, or a mixture of proteins, one of which belongs to this group. It differs considerably from most other globulins in its solubilities, but appears to be biologically identical with the seroglobulin of the lactating animal.[15]

The rapid growth of the young on a milk diet indicates that the proteins of milk are of excellent nutritive quality, and this is borne out by the experience of many investigators.

Thomas[16] calculated what he termed the "biologic values" of different proteins by finding the minimum amount of each necessary to prevent loss of tissue protein from the body on a diet of starch and sugar. Assigning the value 100 to milk proteins, the proteins of other common foods can be arranged in the following order on the same scale:

Milk	100	Yeast	71
Ox Meat	104	Casein	70
Fish	95	Peas	56
Rice	88	Wheat flour	40
Potatoes	79	Corn meal	30

Thomas' results have been widely quoted, but are apparently not entirely reliable.[17] More valuable evidence is offered by later experimenters.

McCollum[18] gives the following table showing the lowest plane of protein intake derived from a single seed which just suffices to maintain an animal in body weight, when the factors other than protein are properly constituted:

Source of Protein	Plane of Protein Necessary for Maintenance
Milk	3.0 per cent of food mixture
Oat (rolled)	4.5 " " " " "
Millet seed	4.5 " " " " "
Maize	6.0 " " " " "
Wheat	6.0 " " " " "
Polished rice	6.0 " " " " "
Flax seed	8.0 " " " " "
Navy bean	12.0 " " " " "
Pea	12.0 " " " " "

14 **Osborne and Wakeman**, J. Biol. Chem. 33, 13, 1918.

15 See **Lane-Claypon**, Oleomargarine, p. 37.

16 **Thomas**, Archiv. fur. Physiol. 1909, 219.

17 **Hindhede**, Skand. Arch. Physiol. 31, 259, 1914; **Sherman**, J. Biol. Chem. 41, 97, 1920.

18 **McCollum**, "Newer Knowledge of Nutrition," N. Y. 1919, p. 75.

These maintenance experiments were of three to six months' duration.

The value of milk or the separate milk proteins as a supplement to cereal grains in animal husbandry has been repeatedly emphasized. In determining the relative values for milk production of different supplements, **Hart** and **Humphrey**[19] found that milk proteins had a percentage efficiency of about 60; oil meal, 61, distillers' grains, 60, gluten feed, 45, corn, 40, and wheat, 36. Later, **Hart** and **Steenbock**,[20] on the basis of their experience with swine, stated that either the cereal proteins, a protein mixture from cereals and alfalfa, or a mixture of proteins derived from cereals, cabbage, and potato, could be effectively supplemented by additions of either milk or whey or meat or fish proteins, milk or whey proteins being the most satisfactory for the purpose. A mixture of proteins from corn, alfalfa, and whey, in which only 16 per cent of the protein nitrogen came from the whey, was nearly equal in value to a similar mixture in which 27 per cent of the nitrogen came from skim milk instead of from whey. The proteins from tankage (animal residues from meat packing) were not quite so effective in their supplementary relations as those from either milk or whey, although the inferiority was slight. A protein mixture drawn from five common sources, rice, wheat, corn, potatoes, and cabbage, with the addition of sufficient wheat gluten to supply 36 per cent of the total nitrogen, showed a production value of 19.4 per cent; that is, from 100 pounds of protein, 19.4 were retained for growth. When the nitrogen from the wheat gluten was displaced by an equal amount of nitrogen from meat crisps the efficiency rose to 32.7 per cent, and when it was replaced by an equivalent in milk nitrogen it rose to 47 per cent.

Recently, **Hart** and **Steenbock**[21] have asserted that when milk is used as a supplement in corn feeding, a highly efficient protein mixture is obtained only when the proportion of liquid milk to corn meal reaches 1:1.

With regard to the practical application of these observations **Sherman** states:[22] "It is plainly desirable in all cases that grain products be supplemented by milk products, and it is clear that in providing for the needs of growing children and of pregnant or nursing mothers, the proportion of milk in the diet should be more liberal than it need be when only maintenance is concerned; this both be-

[19] **Hart and Humphrey**, J. Biol. Chem. 21, 239, 1915; 26, 457, 1916.

[20] **Hart and Steenbock**, J. Biol. Chem. 38, 267, 1919.

[21] **Hart and Steenbock**, J. Biol. Chem. 42, 167, 1920.

[22] **Sherman**, J. Biol. Chem. 41, 97, 1920.

cause of the superior amino acid makeup of the milk proteins and to provide amply for the mineral elements and vitamins as well."

Osborne and **Mendel**[23] endeavored to ascertain the nutritive value of the separate milk proteins as compared with certain vegetable proteins by careful experiments with rats, in which the experimental animals were all of the same sex, and the same initial body weight, and the same total food intake was furnished daily in precisely equivalent and increasing amounts in successive periods of the experiment in accord with the anticipated needs of increasing body weight. The diets used were made up of purified protein, 8.0 to 20.45 per cent, "protein-free milk," 28 per cent, starch and sucrose, 23 to 36 per cent, butter-fat, 18 per cent, lard, 7 to 12 per cent. Comparing lactalbumin, casein, and edestin (a vegetable protein), lactalbumin was found to be far superior, 50 per cent more casein and nearly 90 per cent more edestin being required to produce the same gain in body weight. Good growth was secured with as little as nine per cent of lactalbumin in the ration. The superiority of lactalbumin for maintenance as well as for growth was shown by tests with adult animals. It was found that casein could, however, be supplemented by the addition of a particular amino acid, cystine, in which it was known to be lacking, so as to render it equal to lactalbumin.

On the other hand, in a later set of experiments[24] in which the ration used was purified protein, 18 per cent, "artificial protein-free milk,"[25] 29.5 per cent, starch, 16.5 to 26 per cent, butter-fat, 18 per cent, lard, 7 to 16 per cent, and yeast, 1.5 to 2 per cent, while the rats grew normally when the protein was casein they usually failed when certain vegetable proteins were given, and almost invariably when lactalbumin was fed. It would seem, therefore, that the natural "protein-free milk" used in the earlier experiments provided something necessary to supplement the lactalbumin which was lacking in the later experiments. **Osborne** and **Mendel** say: "Whatever the nutritive value of the unknown constituents of milk may be, our experience manifestly leads to the conclusion that the supplementary value of "protein-free milk" in the diet is, as a general rule, decidedly greater than that of yeast."

McCollum, Simmonds, and **Parsons**[26] obtained somewhat similar results when using a basal ration of 45 per cent dried peas, 9 per cent

[23] **Osborne** and **Mendel,** J. Biol. Chem. 20, 351, 1915; 26, 1, 1916; 37, 223, 1919.

[24] **Osborne** and **Mendel,** J. Biol. Chem. 31, 155, 1918.

[25] See p. 62.

[26] **McCollum, Simmonds,** and **Parsons,** J. Biol. Chem. 37, 287, 1919.

purified protein, 38.2 per cent dextrin, 5.0 per cent butter-fat, and 1.8 per cent salts. Much better growth was secured with casein than with lactalbumin. They stated therefore: "We are forced to the conclusion that lactalbumin is a poorly constituted or an incomplete protein and that the excellent results of Osborne and Mendel were due to the high proportion of nitrogen derived from the 'protein-free milk' which was present in their food mixtures and served to supplement the lactalbumin with respect to some as yet undetermined cleavage product which is essential for growth." **Emmett** and **Luros**[27] found indications of a peculiar relation between lactose and lactalbumin, since a diet made up of lactalbumin (10 per cent), butter-fat, lard, starch, salt mixture, and purified lactose promoted excellent growth, whereas on a diet similar in all respects except that the lactose was replaced by additional starch and alcoholic extract of either wheat germ or yeast, growth ceased. They suggest that lactose, when added to a lactalbumin diet, may either overcome some toxicity of the lactalbumin molecule or carry a vitamin other than that found in wheat germ or yeast.

Sure[28] obtained evidence that lactalbumin is an incomplete protein which must be supplemented by the addition of nitrogen compounds which are present in the "protein-free milk" before it is adequate for either growth or maintenance. When lactalbumin was fed as 12 and 18 per cent of a ration of which the other components were dextrin, butter-fat, agar-agar, salts and extract of wheat embryo, no growth was obtained unless the lactalbumin was supplemented with one per cent of its weight of the amino acid cystine. With this supplement 12 per cent of lactalbumin gave excellent growth, but if it was reduced to 9 per cent of the diet (the amount represented as adequate by Osborne and Mendel) nutritive failure ensued. If, however, the lactalbumin was still further supplemented by the addition of five per cent of its own weight of a second amino acid, tyrosine, the growth was excellent at this low level of lactalbumin intake. Sure believes that the efficiency of a protein may largely depend on its constitution as well as composition in amino acids, and that chemical analysis of amino acid content may at times be inadequate to explain the nutritive failure or success obtained with certain proteins. Since lactalbumin is reported as having the average percentage of cystine present in other proteins, he suggests that cystine is so oriented in the complex polypeptide chain of lactalbumin that when it is hydrolyzed by the enzymes in the digestive tract it is split into simpler peptides of such

[27] **Emmett** and **Luros**, J. Biol. Chem. 38, 147, 1919.

[28] **Sure**, J. Biol. Chem. 43, 457, 1920.

form that a great part of them escape further cleavage, and are therefore de-aminized and converted into urea. In view of the results of Osborne and Mendel above referred to, he is of the opinion that protein-free milk supplies the necessary tyrosine (a hypothesis which is confirmed by qualitative tests on "protein-free milk") and also either carries cystine as part of its nitrogen of unknown source, or contains other forms of organically bound sulphur which the animal readily transforms into cystine. An analysis of protein-free milk showed a sulphur content of 0.2 per cent, of which the greater part was in organic combination.

Logical as this hypothesis seems, however, it offers no explanation of the results of Emmett and Luros in which the efficiency of the lactalbumin was apparently dependent upon the presence of lactose.

According to **Osborne** and **Mendel**,[29] lactalbumin is much more efficacious than casein as a supplement to corn gluten. When as little as one-quarter of the protein in the ration is furnished by lactalbumin and the remainder by corn gluten, a rat is able to make perfectly normal growth, while a similar ration in which as much as one-half of the protein was casein and the remainder corn gluten proved inadequate.

Inasmuch as mother's milk serves as the sole food of young and rapidly growing animals it is generally assumed that it must contain all essential inorganic elements for the optimum growth of that species. **Lane-Claypon**[30] quotes experiments carried on by **Hittcher**[31] which would appear to indicate that the nutritive value of cow's milk for calves can be increased by the addition of certain salts. Boiled milk with suitable salt additions was apparently more nutritious than raw milk. Commenting upon this, Lane-Claypon remarks: "If the addition of salts to mother's milk can bring about such striking differences, then that difference in salt content, which is known to exist between the milks of different species of animals may reasonably be expected to play an important part in the nutritive value of the milk of a foreign species."

With the exception of iron,[32] the various salts appear to be present in greater amount in cow's milk than in human milk. In particular the important elements, calcium and phosphorus, are both greatly in excess in cow's milk.

[29] **Osborne** and **Mendel**, J. Biol. Chem. 18, 1, 1914; 29, 69, 1917.

[30] **Lane-Claypon**, l. c., p. 166.

[31] Kalberfutterungsversuche, Landwirtsch. Jahrb. 1909.

[32] **Soxhlet**, Munch. Med. Wochensch. 28, 1529; 1912.

All three vitamins, *A, B,* and *C,* are present in milk. *A,* as noted above, is associated with the butter-fat, and presumably varies in amount with varying fat content.

There is a surprising divergence of opinion among different authorities as to the proportion of *B* present. **Hopkins** [33] found that 2 cc. of milk per day, or an amount furnishing the equivalent of from one to three or four per cent of the total solids in the ration induced satisfactory growth in rats on a diet of casein, fat, starch, sugar, and inorganic salts. Without the milk there was no growth on this diet. Somewhat comparable results were obtained by **McCollum** and **Davis** [34] who report that as little as two per cent of skimmed milk powder in a ration of polished rice, casein, butter-fat, and salts produced nearly normal growth. On the other hand, **Osborne** and **Mendel** [35] found that a diet of casein, starch, butter-fat, lard, and salt mixture had to be supplemented with as much as 24 per cent of dried whole milk or 16 cc. per day of fresh milk in order to induce rapid growth in rats. That vitamin *B* is the decisive factor is indicated by the success attained when the milk is replaced by small doses of brewers' yeast: 0.2 gm. of dried yeast proved more effective in promoting growth than 15 cc. of milk.

The work of **Hopkins** and **McCollum** and **Davis,** together with the early observations of **Osborne** and **Mendel** on the growth-promoting power of "protein-free milk," gave rise to a wide-spread belief that milk was peculiarly valuable as a source of *B*. The later work of Osborne and Mendel just referred to indicates, however, that this is at any rate not invariably true, and that it is wise to assure safety either by the very liberal use of milk or by the addition to the diet of some other source of *B*.

When tested for its antineuritic power milk shows little efficiency [36] which would appear to confirm the results of Osborne and Mendel, unless we assume the growth-promoting and antineuritic power to be due to two distinct factors.

All evidence goes to show that milk is poor in *C,* although by no means devoid of this vitamin. One hundred to one hundred and fifty cc. per day were required to protect guinea pigs from scurvy, and a

[33] **Hopkins,** J. Physiol. 44, 425, 1912; **Hopkins** and **Neville,** Bioch. J. 7, 97, 1913. Being subjected to criticism by **Osborne** and **Mendel,** who claimed that more than 2 cc. of milk is necessary, **Hopkins** has confirmed his previous work. (Biochem. J. 14, 721, 1921.)

[34] **McCollum** and **Davis,** J. Biol. Chem. 23, 181, 1915.

[35] **Osborne** and **Mendel,** J. Biol. Chem. 34, 537, 1918; 41, 515, 1920.

[36] **Cooper,** J. Hyg. 12, 436, 1912; 14, 12, 1914; **Gibson** and **Concepcion,** Phil. J. Sc. 11B, 119, 1916; **Chick** and **Hume,** J. Roy. Med. Corps, 29, 121, 1917.

little more than this was usually required to afford protection to monkeys.[37] Experience in human nutrition has shown its great inferiority to fresh fruit and vegetable juices in this respect,[38] although it is effective if given in sufficient amount.[39]

Mattill and **Conklin**[40] found that while cow's milk appeared to be entirely satisfactory for the growth of young rats from weaning (when about 25 days old) to about the fiftieth day of life (equivalent to about five years of human life) a decided retardation of growth and sometimes a considerable loss of weight occurred later, usually between the fiftieth and one hundredth days. After this growth might be resumed again, but at a slower rate, and there was no reproduction. Milk powder was somewhat more satisfactory than fluid milk, males growing at a practically normal rate on this, though females were somewhat below normal after about 75 days. This is in accord with the observations of **Lane-Claypon**,[41] who found that rats made slightly better progress on dried milk than on fluid milk. Mattill and Conklin found that reproduction failed on dried milk as on fluid milk. The average daily intake of milk, computed in terms of milk solids, was somewhat less when this was given as fluid than when fed as powder, which may doubtless account for the better progress on the latter food. Addition to the milk of certain inorganic salts was without effect, except in one or two cases where additional iron proved beneficial for growth, but had no effect on reproduction. Addition of yeast or wheat embryo to supply additional *B* increased the rate of growth slightly, but the improvement was only temporary. One female became pregnant on the yeast-milk diet, but the young were eaten. Addition of as much as 10 per cent of butter-fat to the ration of dried whole milk failed to induce continued normal growth, but a ration of 53 per cent dried milk, 5 per cent butter-fat, and 40 per cent starch proved entirely satisfactory for growth. Reproduction took place, but the young were eaten by the mothers. **Osborne** and **Mendel**[42] have, however, reported successful growth, continued in the second generation, on a somewhat similar diet of 60 per cent milk powder, 12 per cent starch, and 28 per cent lard. It would seem therefore that milk lacks something which is essential for growth during part, at least, of the rat's life and for fertility. It is improbable that

[37] **Barnes and Hume,** Bioch. J. 13, 306, 1919.

[38] **Curran,** Dub. J. Med. Sci. 7, 83, 1847; **Barlow,** Lanc. 1894, ii, 1075.

[39] **Comrie,** Ed. Med. J. 24, 207, 1920.

[40] **Mattill and Conklin,** J. Biol. Chem. 44, 7, 1920.

[41] **Lane-Claypon,** J. Hyg. 9, 233, 1909.

[42] **Osborne and Mendel,** J. Biol. Chem. 15, 311, 1913.

this factor is identical with the recognized vitamins, since it is apparently supplied by starch. When the starch is replaced by lactose to the extent of 25 per cent, the remaining 75 per cent of the food being obtained from dried milk, all growth stopped.

Milk powder prepared by spraying milk with heated air has been tested by **Dutcher** and **Ackerson.**[42a] Eight guinea pigs, used as controls, were fed a diet of oats *ad libitum* and 30 c.c. (daily) of fresh raw milk. Ten guinea pigs were fed oats and milk powder (prepared from the same herd milk). The milk powder was diluted back to the same composition as the original raw milk and 30 c.c. were fed daily to each animal. The milk powder was prepared at intervals of 2 to 5 days by spraying milk into a blast of hot air in a cell four feet square. Each quart of milk came in contact with approximately 1,400 cubic feet of hot air. The air in the cell was kept at a temperature of 115° C. while the temperature at the spray nozzle never exceeded 100° C. The milk powder was allowed to remain on the floor of the cell during the drying process (2 to 3 hours). No attempt was made to approximate commercial conditions. The entire group of guinea pigs receiving the milk powder died with pronounced scurvy lesions in periods ranging from 16 days to 42 days. At the end of 42 days all of the control animals, which had consumed their daily ration of raw milk, were living and in much better physical condition than the group receiving the dried milk.[42b]

[42a] **Dutcher** and **Ackerson,** Science, 54, 442, 1921.

[42b] Four series of experiments were carried out by **Jephcott** and **Bacharach** (Biochem. J. 15, 129, 1921) to determine the antiscorbutic potency of dried milk. In the first three, comparison was made of summer milk, winter milk and winter milk neutralized with sodium bicarbonate before drying. All three were dried by the roller process, in which the milk is in contact with the source of heat for not more than three seconds. The fourth variety was full-cream milk dried by the spray process; this variety of milk is subject to prolonged heating. The composition of the four varieties of milk powder varied but little; they each contained in the neighborhood of three-fourths per cent of moisture, 5.5 to 6.5 per cent of ash, 26 to 28 per cent of fat, 35 to 37 per cent of lactose, and 25 to 27 per cent of protein. Guinea pigs were fed with the usual scorbutic diet of oats and bran, and the milk was added in the form of a thin cream by mixing the powder with three times its weight of hot water. The results may be summarized as follows:

	Summer milk	Winter milk	Neutralized milk	Spray milk
Under experiment..........	9	11	4	12
Developed scurvy..........	2	3	3	12
Survived, or died of causes. other than scurvy........	6	9	1	..

The question of how far the proportions of the relative constituents present in milk can be varied by alteration in the diet of the lactating animal has received much attention from investigators, especially in connection with the vitamin content.

It is the general experience of practical dairymen and of investigators in the field of animal nutrition that diminution in the amount of food below the physiological requirements of the animal results in decrease in the amount of milk secreted, and that increase in food causes increased production of milk, although the increase is not proportional to the amount of food given.[43] The effect of underfeeding is less marked in the early stages of lactation than later. **Eckles** and **Palmer** report a case in which a constant flow of milk was maintained for 30 days by an animal receiving only sufficient food for body maintenance.[44] When the lactating period has reached a certain stage, however, even moderate underfeeding reacts adversely upon the milk supply. **Lane-Claypon**[45] asserts that it is probable that many cases of failure of lactation in women have been caused by the absence of proper nourishment for the mother, and quotes the experience of institutions in which dinners are provided for nursing mothers. "It is the opinion of the officers who supervise the work at these centres that the provision of an adequate food supply for the mother almost invariably enables breast-feeding to be maintained, mothers who have previously been unable to feed their children being able to do so without difficulty when provided with sufficient food of a suitable nature. Moreover, in many instances, where the milk supply was apparently becoming short, it has been completely regained and lactation continued up to the ninth month. No special foods or substances are either indicated or contra-indicated. The health of the mother must be maintained. Where this is effected owing to the idiosyncrasy of the individual for any particular foodstuff, that foodstuff should be avoided — that is all."

On the other hand, **Ducaisne**[46] has called attention to the fact that during the siege of Paris healthy young women were able to produce

---

These results are not in accord with those obtained by several other investigators.

Stepp notes that accessory food substances are present in dried milk powders (Med. Klin. 17, 287, 1921).

[43] See **Lane-Claypon**, Milk and its Hygienic Relations, London, 1916, p. 20; **Eckles** and **Palmer**, Mo. Agr. Exp. Sta. Res. Bulls. 24 and 25.

[44] See also **Hart**, quoted by **McCollum**, **Simmonds** and **Pitz**, J. Biol. Chem. 27, 33, 1916.

[45] **Lane-Claypon**, l. c., p. 157.

[46] **Ducaisne**, Gaz. Med. Paris, 1871, 317.

enough milk for the maintenance, and in some cases for growth, of their infants while partially fasting themselves.

While the character of the food of the animal doubtless influences the richness of the milk to some extent in the long run, it seems to affect the quality of the milk produced less than its quantity. According to **Woll**,[47] although high feeding may produce an increase both in the amount of milk and in the butter-fat this rise is in no way proportional to the increased amount of food.

Eckles and **Palmer**[48] state that the effects of an induced subnormal plane of nutrition on the percentage of fat in the milk are variable, an increased fat percentage resulting in some cases, with no change in others, and in still others an actual decrease in the percentage of fat. The factors that appear to cause these variations are the state of flesh of the animal, the degree of underfeeding, and the season of the year in which the experiment is conducted.

In the case of physiological underfeeding there is almost invariably an actual increase in the yield of milk fat, as well as in the percentage in the milk. The character of the milk fat is altered, as shown by the change in the physical and chemical constants. Eckles and Palmer suggest that the production of the normal amount of milk fat is controlled by the activity of the lipases and other enzymes which accelerate this synthetic reaction in the mammary gland, being greatly influenced by changes in the general metabolic activity of the body, particularly by the changes that affect the fat metabolism, and that the composition of the fat produced is controlled by variations in the amount and kind of material presented to the milk glands by the blood stream. **Koning**,[49] on the other hand, found that poor food caused a fall in the fat content of the milk. **Behr**[50] states that owing to the deficiency of food, the average fat content of milk in Germany fell to 2.98 per cent in 1918, being a gradual decline since 1914. The quantity of milk fell also from 2900 l. to about 1700 l. per year.

Engel and **Plaut**[51] found that the milk of a nursing mother is reduced if the diet is deficient in fat, but was unable to increase the fat in the milk by increasing the fat in the food.

According to **Eckles** and **Palmer** (l. c.) a subnormal plane of nu-

[47] **Woll**, Agric. Exp. Sta. Wisconsin, Bull. 116.

[48] **Eckles** and **Palmer**, Agric. Exp. Sta. Un. Mo. Bull. 25, 1916.

[49] **Koning**, Milchw. Zentr. 6, 675, 1910.

[50] **Behr**, Z. Nahr. Genussem. 35, 471, 1918; 37, 165, 1919.

[51] **Engel** and **Plaut**, Mun. Med. Wochensch. 55, 1158, 1906, quoted by **Lane-Claypon**, Milk and its Hygienic Relations, London, 1916.

trition at times affects the percentage of protein in the milk. In some of the experiments conducted there was a decline in the percentage of casein, while in others the total protein only was affected. In the latter cases the percentage of ash in the milk was decreased. None of the types of underfeeding influenced the percentage of lactose in the milk.

Many attempts have been made to increase the mineral content of milk by additions of salts to the food, particularly in the case of iron [52] and calcium,[53] but there is no decisive evidence of appreciable success.[53a] **Kohn** [54] found that both the ash and the fat content of goats' milk was higher when the animals were grazing in the fields than when they were restricted to dried feed. **Fingerling** [55] found that when goats were fed on a diet deficient in calcium and phosphorus a decrease both in the total amount of milk and in the proportion of these constituents occurred, but only after a certain period had elapsed, during which the system became gradually depleted. On addition of the missing elements to the diet the secretion became normal. **Hess, Unger,** and **Supplee** [56] have noted that the percentages of calcium and phosphorus were below normal in milk produced on a ration of bean meal, oil meal, bran, beet pulp, molasses, and straw, which contained an ample supply of minerals, but was entirely deficient in vitamin *C*. **Koning,**[57] and **Allemann** [58] found that even large increases of sodium chloride in the food caused no rise in the salt content of the milk.

Babcock [59] fed cows on a ration suitable in every respect except that the sodium chloride supply was limited to that naturally present

[52] **Lane-Claypon,** l. c., p. 53.

[53] **Zuckmayer,** Pflug. Arch. f. d. ges. Physiol. 158, 209, 1914; **Dibbelt,** Arb. a. d. Geb. d. path. Anat. u. Bakt. Tub. 1908; **Schabad,** Jahrb. f. Kinderh. 74, 511, 1911.

[53a] **Rumsey** (Brit. Dental J. 42, 49, 1921; Physiol. Abstracts 6, 66) notes that the incidence of caries is complex, and diet a most important factor. He considers milk to be the ideal food for supplying the necessary calcium and should be relied on during the growth-period; bread should be made so as to contain more cellulose and more vitamin; among fats, olein is preferable to the more solid fats; calcium intake should be regulated in adults, and symptoms of thyroid abnormality should be recognized and treated.

[54] **Kohn,** Deutsch Tier. Wochenscht. 21, 49, 1913.

[55] **Fingerling,** Landwirtsch. Versuchsstat. 75, 1, 1911.

[56] **Hess, Unger,** and **Supplee,** J. Biol. Chem. 45, 299, 1920.

[57] **Koning,** Milchw. Zentr. 6, 675, 1910.

[58] **Allemann,** Schweizer Milchwent. 1911, Nos. 67, 71, 72, 74, quoted by **Lane-Claypon,** l. c. p. 21.

[59] **Babcock,** Wis. Exp. Sta. Ann. Rep. 22, 1905.

in the food, until some of the animals died, and others were only saved from death by the administration of salt. In no instance was there notable decrease in the yield of milk until just before general decline set in, but the ash content of the milk under these circumstances was not reported.

McCollum and Simmonds[60] made an elaborate study of the relation between the diet of lactating rats and the value of the milk produced. The rats were fed on an adequate diet until the birth of the young, when they were immediately placed upon a ration known to be entirely inadequate for growth owing to deficiency in one or more factors. The litters were in all cases reduced to four, so that the burden imposed upon the mothers was uniform in this respect. Their results show that the mothers were capable of providing milk which was better adapted for growth than the diet which they themselves received; but sooner or later failure ensued. On a diet of rolled oats, deficient in three factors, protein, mineral content, and vitamin *A*, the young grew very slowly for the first 20 days, then became stunted and died between the fortieth and fiftieth days. When *A* was added to this diet the young grew faster and growth continued over a longer interval, up to 50 days. Although they began to eat the oat and butter-fat diet of the mother about the twentieth day it is evident that this must have been supplemented by the inorganic content of the milk, since without such supplement rolled oats fails entirely to support growth. Addition of protein was of less benefit than addition of salts. When the mother was fed on a ration of rolled oats supplemented with protein only, the best growth observed was half-normal. When *A* was applied in addition the young did slightly better, but addition of mineral salts was more beneficial than addition of *A*. Only when all three deficiencies, *A*, salts, and protein, were corrected was it possible to secure entirely normal growth, but in every case after the young were old enough to share in the mothers' diet they grew for a time, although slowly, which would have been impossible on this diet alone, showing that the mother was producing milk capable of supplementing to some extent the diet on which that milk was produced. According to McCollum and Simmonds, the ultimate failure which invariably ensued indicated that the milk produced on these deficient diets was not of normal quality.

With respect to the vitamins, it is the general opinion that these pass into the milk only as supplied from the food of the mother, and

[60] **McCollum and Simmonds,** Am. J. Physiol. 46, 275, 1918; **McCollum,** "Newer Knowledge of Nutrition," 1919, pp. 117–185.

that the mammary gland has no power of synthesizing these essentials.[61]

Since *A* is present to some extent in the body fats of animals it is probable that small amounts of this will be secreted in the milk, for a time at least, even if it is not supplied in the food, but the experience of McCollum and Simmonds above referred to, indicates that under these circumstances it is not sufficiently abundant for the optimum growth of the young. **Hopkins**[62] found that the vitamin *A* of goat's milk remained approximately constant when the animal was given a diet deficient in that factor, although the quantity of milk fell off, but the length of the experimental period is not stated.

If we accept the hypothesis that rickets is connected with deficiency of *A*, then the observation of **Hess**[63] as to the prevalence of rickets among the breast-fed children of the negro women whose diet is limited almost exclusively to substances poor in *A*, must be regarded as significant.

Dutcher, Kennedy and **Eckles**[64] have found that spring milk, produced when the cows are pasturing on fresh grass, is superior to winter milk from cows on dry feed with regard to both *A* and *B*. The tests were made by comparing the growth of rats on a ration of casein, dextrin, agar agar, butter-fat, extract of wheat embryo, and an adequate salt mixture, with that of other groups in whose diets either the butter-fat or the wheat embryo had been replaced by varying quantities of winter milk or spring milk. When the spring milk was at its best 10 cc. per day furnished sufficient of both *A* and *B* for normal growth, but butter-fat from winter milk had to be fed to the extent of 20 per cent of the ration in order to secure growth, and even this amount was not always adequate.[65]

Osborne and **Mendel**[66] were unable to observe any difference between summer and winter milk in the relative amounts of *B* present, which was perhaps to be expected from the relative stability of this vitamin and its wide distribution in the grains which make up a part of the ordinary winter feed of dairy cows. **Dutcher, Eckles, Dahle, Mead,** and **Schaefer**[67] state, however, that in their experience the content of *B* in the milk of cows is influenced by the character of the

[61] See **McCollum, Simmonds** and **Pitz**, J. Biol. Chem. 27, 33, 1916.

[62] Quoted by **Harden**, J. Soc. Chem. Ind. 40, 79R, 1921.

[63] **Hess**, J. Am. Med. Assoc. 70, 900, 1918.

[64] **Dutcher, Kennedy,** and **Eckles**, Proc. Am. Chem. Soc. Sci. 52, 588, 1920.

[65] See also **Hess** and **Unger**, J. Am. Med. Ass. 74, 2171, 1920.

[66] **Osborne** and **Mendel**, J. Biol. Chem. 41, 515, 1920.

[67] **Dutcher, Eckles, Dahle, Mead,** and **Schaefer**, J. Biol. Chem. 45, 121, 1920.

ration, although less markedly than is true of the vitamins *A* and *C*, and **Andrews** [68] has shown that nursing mothers on a diet deficient in antineuritic *B* produce milk on which their nurslings develop beriberi.

Several investigators have reported a seasonal variation in the antiscorbutic properties of cow's milk,[69] and this point may be regarded as established. **Hart, Steenbock and Ellis** found that while 50 cc. per day (and in some cases less) of summer pasture milk afforded protection to guinea pigs against scurvy, at least 75 cc. of winter feed milk were required to provide the same degree of protection. They remark the fact that the milk produced from such dry foods as are used does contain a measure of antiscorbutic vitamins shows that the dry feeds themselves are not completely devoid of this factor. " Their content in this vitamin is too low for protection of a guinea pig against scurvy, but the mammary gland, through a process of special selection and concentration in the milk produced becomes an effective means of showing the presence of *C*, especially in old dried feeds." Neither silage nor sugar mangels were notably effective in increasing the proportion of antiscorbutic in the milk. **Barnes** and **Hume** [70] suggest that the use of turnips rather than mangels should result in the production of milk of higher antiscorbutic value.

Dutcher and his collaboraters found that there is a decided tendency for the nutritive properties of the milk to remain relatively good for four to eight weeks after the cow has been placed on a vitamin-poor winter ration. On the other hand, the nutritive superiority of spring milk became evident almost immediately after the cows were placed upon green grass.

The question of the effect of heat on the nutritive value of milk is of the greatest practical importance in an age when the use of pasteurized, condensed, evaporated, and dried milks is constantly increasing, and raw milk of good quality is increasingly difficult to secure.

The pasteurization of milk is usually carried out either by heating the milk rapidly to a point not far short of boiling, maintained there for a few seconds and then cooled as rapidly as possible (the " flash "

[68] **Andrews**, Phil. J. Sci. 7B, 67, 1912.

[69] **Barnes and Hume**, Lanc. 1919, ii, 323; **Dutcher, Eckles, Dahle, Mead,** and **Schaefer**, J. Biol. Chem. 45, 119, 1920; **Hart, Steenbock and Ellis**, *Ib.*, 42, 383, 1920; **Hess, Unger and Supplee**, *Ib.*, 45, 229, 1920.

[70] **Barnes and Hume**, Bioch. J. 13, 306, 1919.

method of pasteurization), or by heating to 60° to 63° C. (140° to 145° F.) and holding at this temperature for 20 to 30 minutes or even longer (the "hold" method). The temperatures used may vary considerably from those mentioned but the essential difference between the two methods lies in the fact that in the one the milk is heated rapidly to a high temperature and cooled immediately, while in the other it is held at a lower temperature for a much longer period. The "hold" method is usually recommended as preferable on account of the lower temperature involved, but the work of **Daniels** and **Loughlin** referred to below (p. 282) indicates that this is a mistaken point of view. The "hold" process is the one most generally employed commercially in the United States, while the "flash" process is more generally used in Great Britain.

While pasteurization serves to destroy the most harmful of the disease bacteria as well as the lactic acid bacilli which cause souring, certain bacterial forms are capable of surviving this process, notably the bacteria which bring about putrefactive decomposition of milk. Pasteurized milk may therefore deteriorate on keeping quite as seriously as raw milk; in fact it may be more actively injurious although the change is not made evident by the development of a sour taste.

Sterilization of milk results in the destruction of all the bacteria present, and is carried out by heating to a temperature of 220° to 230° F. under pressure in an autoclave. The term is often, although incorrectly, applied to boiled milk. Boiling does not ensure complete sterilization, unless prolonged for 30 minutes or more, but milk which has been boiled for a much shorter time may be regarded as quite safe for any ordinary purpose, provided it is protected from contamination and used within a reasonable time after treatment.

The early experiments with animals of various species fed upon heated cow's milk are summarized by Lane-Claypon, but offer little of interest, as in most cases the diet was entirely unsuitable apart from the possible deterioration on heating.

Experiments with calves fed on boiled and sterilized milk have given somewhat contradictory results. Certain American investigators [71] have reported unfavorably on its use, while **Dean** [72] recommended it. Danish experience indicates that milk from tuberculous cows can be used successfully for feeding calves.[73]

There is a firmly established tradition in many households that

[71] **Doan** and **Price**, Maryland Agric. Exp. Sta. Bull. 77, 1901; **Price**, N. Y. Med. J. 79, 405, 1904.

[72] **Dean**, Rep. Ont. Dept. Agric. 1898, i, 66.

[73] **Bang**, quoted by Lane-Claypon, l. c.

heated and especially boiled milk is highly injurious to children and more or less deleterious for adults as well, the reason usually given being that " it tends to induce constipation." Several eminent children's specialists have strenuously opposed pasteurization of milk for babies on the ground that the heating injures its nutritive properties. On the other hand, there is much clinical evidence that boiled milk is if anything superior to raw milk.[74] In European countries where wet nurses are rather extensively used it is quite common to have the milk of these nurses boiled in order to lessen the risk of transmission of disease, and there is no evidence that the value of the milk is impaired in any way by this procedure. Reports from children's hospitals go to show that there is comparatively little difference as far as nutritive power goes, between raw and boiled milk, and advantage being on the side of the boiled milk if anything.[75] There appears to be absolutely no basis for the belief that boiled milk is more constipating or less digestible than raw milk. While occasional cases are reported in which sick children have apparently done better on raw than on boiled milk these are counterbalanced by at least an equal number of cases in which boiled milk has proved more efficient than raw. In a recent series of experiments with guinea pigs, boiled milk was found to be practically equivalent in nutritive value to unheated milk.[76] **Park** and **Holt** [77] made a comparative study for two successive summers on groups of infants from New York tenements fed upon raw and pasteurized milk. The milk used was of good quality and modified similarly for all the children. Seventy-five and six-tenths per cent of the children in the group fed on pasteurized milk remained in good health during the entire summer, while only 33.3 per cent of those on raw milk were equally healthy. Thirteen out of the 51 children in the latter group had to be transferred to pasteurized milk during the experiment on account of severe attacks of diarrhoea.

A form of sterilized milk containing vitamins is prepared by **Hort.**[77a] The milk is sterilized in an autoclave, so arranged that the steam from the outer vessel passes through the milk in the inner vessel before escaping. The autoclave is maintained at 20 pounds per square inch steam pressure (about 116° C.) and passage of steam from 3 to 5 minutes is sufficient to sterilize the milk. Taste, odor, vitamin-content, and emulsified condition of the fats are stated to be unaltered. Sterilized sugar may be added, to make good any loss brought about

[74] See Lane-Claypon, Oleomargarine, Ch. 9–11.

[75] **Finkelstein**, Therap. Monats Oct. 1907; Lane-Claypon, Local Govt. Bd. Rep. No. 63, 1912.

[76] **Anderson, Dutcher, Eckles,** and **Wilbur**, Sci. 53, 446, 1921.

[77] **Park** and **Holt**, Arch. Ped. 1903.

[77a] **Hort**, British Patent 166,984, 1920.

by heating. Cream is prepared from unsterilized milk and then sterilized; butter is made from sterilized milk, and condensed milk is sterilized after condensation.

With regard to the separate constituents of milk, there is considerable evidence to show that the digestibility of caseinogen is increased by heating. **Brennemann**[78] was able to demonstrate that the curd formed from raw milk in the human stomach is very tough and large, while that formed from heated milk is soft and much finer in character.

McCollum and **Davis**[79] found that while milk powder in quantities as small as two per cent of the food mixtures consisting otherwise of polished rice, purified casein, salts, and butter-fat, gave enough *B* for growth, milk powder which had been heated four hours in a double boiler was noticeably less efficient than the unheated product, and after heating for one hour in the autoclave it lost its growth-promoting power almost entirely. Since wheat embryo can be heated to this temperature and for this length of time without lowering its efficiency, McCollum concluded that the loss of efficiency of milk was due to the effect of heat on some factor other than *B*. He therefore made a series of feeding experiments in which a ration deficient only in protein and *B* was supplemented by various preparations from milk which had been exposed to high temperatures; whey, evaporated to small volume at or near boiling temperature and then heated in an autoclave at 15 pounds for an hour was apparently not affected by the heating and still contained sufficient *B* to promote good growth, as did also " protein-free milk " which had been boiled for six hours, and lactose which was heated at 15 pounds for an hour. On the other hand, moist casein which had been heated for an hour at 15 pounds was much less satisfactory for growth than an equal quantity of unheated casein. McCollum and Davis suggest that this may be associated with the loss of sulphur by casein on heating, as demonstrated by **Rettger**[80] and **Morner.**[81] In view of this apparent instability of casein when exposed to heat **McCollum** and **Davis**[82] questioned the desirability of purifying casein for nutrition experiments by extraction with hot alcohol, and devised an alternative method which did not involve heating. **Funk** and **Macallum**[83] could find no difference

78 **Brennemann,** J. Am. Med. Ass. 60, 575, 1913.
79 **McCollum** and **Davis,** J. Biol. Chem. 23, 247, 1915.
80 **Rettger,** Am. J. Physiol. 6, 450, 1901-2.
81 **Morner,** Ztschr. f. Physiol. Chem. 34, 207, 1901-2.
82 **McCollum** and **Davis,** J. Biol. Chem. 23, 231.
83 **Funk** and **Macallum,** J. Biol. Chem. 27, 51, 1917.

in the nutritive qualities of casein as prepared by the method of McCollum and Davis or by the usual method of extraction. They confirmed the statement of McCollum and Davis as to the loss of efficiency of casein on heating in the autoclave for an hour, but found that the fault could be corrected by addition of 1 cc. of orange juice, from which they concluded that rats, in spite of their unsusceptibility to scurvy, require a certain amount of the antiscorbutic vitamin in their food, and that the deterioration of the casein was due to the destruction of this factor rather than to the alteration in the character of the protein itself. Their results are of questionable value, however, since the rations used were deficient in *A*.

Hogan,[84] using rations of butter-fat, starch, agar, protein-free milk, and casein, obtained results at variance with those of McCollum and Davis. Even when the casein had been heated for two hours at 45 pounds pressure its nutritive value was unimpaired.

Emmett and **Luros**[85] suggest that the discrepancy between the observations of Hogan and of McCollum and Davis may be due to a growth-promoting vitamin which is supplied in the protein-free milk and in the unheated casein but is destroyed by heat. These investigators tested lactalbumin which had been heated in an air oven at 90° to 100° C. for 16 hours, or in an autoclave at 15 pounds pressure for six hours, and found no alteration in its growth-promoting power.

Lane-Claypon[86] states that: "It seems certain that when milk is boiled some of the albumin is precipitated, but where the same vessel is used for storage and feeding purposes, there is no difficulty in maintaining the same amount of nitrogen in the food. There is no indication that there is any difficulty in the digestion of the coagulated albumin; in fact it is probably more readily digested than in the raw state."

It is a well-known fact that the calcium content of milk is reduced on heating, owing to the precipitation of insoluble calcium salts. **Daniels** and **Loughlin**[87] in the course of an investigation into the nutritive defects of heated milk obtained some very interesting results which seem to indicate that this precipitation of calcium may be the primary cause in rendering heated milk insufficient for the nutrition of certain animals. It is improbable that this would have any effect in infant feeding, since cow's milk contains considerable excess of calcium as compared with human milk.

[84] **Hogan**, J. Biol. Chem. 27, 193, 1916; 30, 115, 1917.
[85] **Emmett** and **Luros**, J. Biol. Chem. 38, 257, 1919.
[86] **Lane-Claypon**, l. c., p. 230.
[87] **Daniels** and **Loughlin**, J. Biol. Chem. 44, 381, 1920.

The experimental animals used by Daniels and Loughlin were healthy young rats. It had previously been observed by **Daniels** and **Stuessy**[88] that rats fed on milk which had been boiled for even one minute grew subnormally and never reproduced, but that the addition of well-washed coagulated egg yolk or white or casein induced normal growth, and, in the case of the egg yolk at least, successful reproduction. In these experiments the milk was placed in glass containers holding one pint, surrounded with cold water, and brought to a boil. Since the length of time the milk was held at boiling point (1, 10 or 45 minutes) appeared to make no appreciable difference in the results, in the second set of experiments the milk was brought quickly to the boiling point in an open kettle and then maintained at boiling temperature for one minute. To the surprise of the investigators the rats fed on milk so treated grew normally and appeared perfectly nourished. Repetition of the earlier experiments confirmed the observations first made. It was evident therefore that milk which had been slowly heated to boiling point was not equivalent for nutritive purposes to that which had been heated rapidly to the same temperature. Experiments were then undertaken with milk pasteurized by heating gradually (in the course of 30 to 45 minutes) to 65° and 82° C. respectively, the milk being held at this temperature for 40 minutes and then cooled with running water. Condensed and evaporated milk were also tested. The condensed milk had been prepared by heating to approximately 200° F. and then evaporated in vacuum pans to the desired consistency, the temperature during this part of the operation averaging 150° F., but dropping to about 120° before its completion. In the preparation of the evaporated milk it was held at the boiling temperature for 10 minutes, then evaporated at 130° to 140° F., cooled, canned, and sterilized at 240° F. for about 20 minutes. The pasteurized milk failed to induce more than one-half normal growth, and the animals on the evaporated milk grew very slowly but in a miserable condition for a longer or shorter period and then died. It is noteworthy, however, that they showed no signs of xerophthalmia. Additions of butter-fat or extract of wheat embryo were without effect in stimulating growth, and separate tests on purified rations deficient in *B* but supplemented with fresh and evaporated milk indicated if anything a rather higher content of *B* in an amount of evaporated milk supposed to be the exact equivalent of the fresh milk used. Addition of calcium lactate, however, produced much better growth, both with the evaporated and the slowly pasteurized milk. When calcium glycerophosphate was used instead of calcium lactate the results were

[88] **Daniels** and **Stuessy**, Am. J. Dis. Child. 11, 45, 1916.

even better, growth and reproduction both being highly successful. The best growth of all was obtained when tricalcium phosphate was made into a paste with starch and fed with evaporated milk.

These results are in line with those obtained by **Hittcher**[89] in feeding calves on boiled milk, in which he found that boiled milk with the addition of tricalcium phosphate was much more efficient for growth than raw milk or milk supplemented with any other salt. Daniels and Loughlin suggest that on long heating sufficient insoluble calcium salt precipitates to lower the nutritive value of the milk, a hypothesis which is confirmed by the fact that when especial care was taken to include the insoluble material by incorporating the whole in a paste, results comparable to those on raw milk were obtained. The better growth of the animals on condensed milk as compared with evaporated milk is explained by the fact that the condensed milk which is of a thick viscous consistency was fed undiluted and consequently the insoluble material remained fairly well distributed through it. The improvement noted in the work of Daniel and Steussy on addition of egg yolk and casein would naturally follow from the amount of calcium and phosphorus in these substances. It may be noted in this connection that **Soldner**[90] found a loss of from 13 to 15 mgs. of calcium and 10 to 13 mgs. of phosphorus per 100 cc. of milk which was just brought to a boil. **Solomin**[91] found that at 130° to 140° C. about one-half of the ash of milk, including all of the calcium phosphate, was precipitated.

It seems clear from the foregoing that neither of the vitamins *A*[92] and *B* are appreciably affected by the ordinary heating of milk. It should be noted, however, that **Sekine**[93] found that although weaned white mice thrive well on condensed milk alone for about 100 days, they finally suffer from polyneuritis and anemia. When iron and vitamin *B* were added, the mice became normal.

The antiscorbutic vitamin is more susceptible to heat, and it might therefore be expected that heated milk would show a deficiency in this respect, an expectation which is fully borne out by the reports of various observers.

[89] **Hittcher,** Landwirtsch. Jahrb. 1909, quoted by Lane-Claypon, p. 166.

[90] **Soldner,** Landw. Versuchs. 1888, p. 351, quoted by Lane-Claypon, *l. c.* p. 351.

[91] **Solomin,** Arch. f. Hyg. 28, 43, 1897.

[92] See also **Barnes and Hume,** Bioch. J. 13, 306, 1919.

[93] **Hildesaburo Sekine,** J. Tokyo Chem. Soc. 41, 439, 1920; Chem. Abs. 1920, 2813.

The conflicting reports from practitioners as to the prevalence of infantile scurvy among babies fed upon pasteurized milk may perhaps be reconcilable by the theory of **Hess**, that aging of milk is an important factor in the destruction of C.[94] Hess observed that home pasteurized milk was less likely to induce scurvy than that which had been pasteurized commercially, although the temperature employed was the same in both cases (145° F. for 30 minutes). As the only difference in the treatment given the milk was to be found in the interval of time between pasteurization and consumption he tested the effect of aging by keeping home-pasteurized milk on ice for 48 hours before using. Of eight infants which were fed the milk so treated, two showed scorbutic symptoms which were relieved by giving them orange juice. Two out of another eight which were fed milk that was kept on ice forty-eight hours after the heat treatment, showed signs of scurvy. In other cases scurvy was observed in infants fed certified milk which had not been pasteurized, when the latter had been kept on the ice forty-eight hours before feeding.

The general trend of evidence from animal experimentation goes to show that the antiscorbutic, which is relatively low in cow's milk at best, is decreased by heating, the amount of destruction increasing rather rapidly with increase in temperature. **Frölich**[95] states that milk heated to 70° C. afforded protection to guinea pigs against scurvy, while milk heated to 98° C. for ten minutes failed to do so, but the amounts consumed are not noted. **Chick, Hume, and Skelton**[96] found that the antiscorbutic power of milk is appreciably decreased by heating to 120° C. under pressure, an observation which was confirmed by **Hart, Steenbock, and Smith.**[97] In ordinary pasteurization the milk never reaches so high a temperature as this. **Barnes and Hume**[98] found that a ration of 200 cc. of milk which was brought just to a boiling temperature caused the rapid and complete recovery of a monkey which had developed scurvy on a daily ration of 200 cc. of dried milk. The amount of milk fed was so large that 25 per cent of the antiscorbutic present might have been destroyed by the heating without detection, but these investigators believe that the rapidity of the cure indicated that the ration contained considerably more than the minimum necessary to effect a cure.

Anderson, Dutcher, Eckles and Wilbur[99] found that pasteurized

[94] **Hess**, Am. J. Dis. Child. 14, 337, 1917.
[95] **Frölich**, Ztsch. f. Hyg. u. Infekt. 72, 155, 1912.
[96] **Chick, Hume and Skelton**, Bioch. J. 12, 131, 1918.
[97] **Hart, Steenbock, and Smith**, J. Biol. Chem. 38, 305, 1919.
[98] **Barnes and Hume**, Bioch. J. 13, 306, 1919.
[99] **Anderson, Dutcher, Eckles and Wilbur**, Sci. 53, 446, 1921.

milk, heated at 145° F. for 30 minutes, produced scurvy very quickly and all of the animals died in a very short time, while boiled milk was practically equal, in nutritive properties, to the unheated raw milk. Examination revealed the fact that the pasteurized milks had been stirred rather violently with motor-driven propellers, while the boiled milk had not been stirred mechanically. This suggested that oxidation had occurred in the pasteurized milks, a theory which was confirmed by subsequent experiments. Some destruction occurs when air is bubbled through milk at 145° F for 30 minutes, but the destruction is much more marked when oxygen or hydrogen peroxide is used. Oxygen and hydrogen peroxide will destroy the antiscorbutic accessory at room temperature although the destructive action is hastened as the temperature increases.[100] Milk may be pasteurized in closed vessels or boiled in the open air without appearing to lose its nutritive and antiscorbutic properties when fed to guinea pigs. When carbon dioxide is bubbled through the milk, it compares very favorably in nutritive properties with the raw milk. Since it is probable, however, that some slight destruction of *C* may take place even at quite low temperatures it would seem wise to take the precaution of supplementing heated milk with orange juice or some other source of *C*, and also, in view of Hess's observations, to shorten the interval between pasteurization and consumption as far as practicable. In this connection Hess's remarks[101] may be quoted:

> It is not to be inferred from these conclusions that the use of pasteurized milk is fraught with danger, but merely that it is an incomplete diet for babies and must be given with antiscorbutic food. Its use is highly desirable and to be recommended for pasteurization does not seem to affect the nutritional value of the milk and renders marked service in preventing the occurrence of various infectious diseases. There are secondary factors in the causation of scurvy. In the first place there is an individual variation which must be taken into account. This would seem to depend upon hereditary characteristics, upon the amount of antiscorbutic material which the infant brings with it when it comes into the world. Secondary food factors would also seem to play a part; in our experience those infants which received malt preparations seemed to be most predisposed to the development of scurvy, and it is quite possible that there is a relationship to carbohydrate diet, similar to that which Funk has drawn attention to in the case of beriberi. Whenever the connection of pasteurization and scurvy is brought forward, the statistics of various French writers who claim to have fed some thousands of babies on sterilized milk without encountering scurvy, are cited in refutation of the harmful effects of heat. Without analyzing these results, it may be said that these infants were cared for in an ambulatory service and that their diet was therefore not under close observation and probably they were not observed for a sufficiently long period to detect the rudimentary form of scurvy.

[100] See also **Hess**, J. Am. Med. Ass. 76, 693, 1921.

[101] **Hess**, 2nd Pan. Am. Sci. Cong. Sec. 8, Pt. 2, p. 49.

What has been said of pasteurized milk will hold in general for evaporated and condensed milks. The general methods of preparation of these products have been described (p. 277). Condensed (sweetened) milk is subjected to about the same degree of heat as that used in the "hold" method of pasteurization, while evaporated milk is usually sterilized by heating under pressure. Both products are free from bacterial contamination but are probably deficient in *C*, and should therefore be supplemented by additional supplies of this vitamin.[102] **Hess**[103] states that sweetened condensed milk has been found to retain the larger part of its antiscorbutic factor, while on the other hand **Giorgi**[104] has described an epidemic of scurvy coincident with the introduction of condensed milk. This contradiction may perhaps be reconciled by the statement of **Hume**[104a] that there is no destruction of vitamin in full-cream sweetened condensed milk prepared by concentration at a low temperature in vacuo, so that if the condensed milk is diluted to its original volume by addition of three volumes of water the vitamin content may be said to be adequate, but that if it is diluted eight times or more, according to instructions, its vitamin content may be inadequate, although, owing to its higher sugar content, it still gives an adequate supply of calories.

The nutritive value of dried milk has been very fully reported on by **Coutts and Winfield.**[105] There are various methods of preparing this product, in some of which the milk is evaporated at a comparatively low temperature, while in others it is exposed to a high temperature, but for a very short time. Usually the milk is either passed in a thin layer over the surface of a heated cylinder, evaporation being hastened by a current of hot air, or forced, after partial evaporation, in the form of a very fine spray, into hot dry air. As an example of the first process, that of **Just-Hatmaker** is described by Coutts as follows:

> Two hollow metal cylinders arranged to revolve in opposite directions are mounted so as almost to touch one another. At one time the process was carried out with the cylinders performing six or seven complete revolutions per minute, but the speed has been increased in later models to 14 times a minute, or even faster. The cylinders are heated internally by steam at a pressure of two to three atmospheres, so that their surface temperature is considerably over 100° C.
>
> The liquid milk runs from an elevated container into the hollow between the two revolving cylinders, the result being a constant rapid ebullition leading no

[102] See **Hart, Steenbock and Smith,** l. c.

[103] **Hess,** J. Am. Med. Ass. 76, 693, 1921.

[104] **Giorgi,** Pediatria, 29, 66, 1921.

[104a] **Hume,** Biochem. J. 15, 163, 1921.

[105] **Coutts and Winfield,** Local Govt. Bd. Food Reps. No. 24, London, 1920.

doubt to a certain concentration of the milk. The milk then spreads in a thin film over the surface of the cylinders, and the water is rapidly evaporated, leaving a thin sheet of dried milk having the appearance of tissue paper. When the cylinders have completed about two-thirds of a revolution, knife-blades, adjusted so as to touch the surface of the cylinders, scrape off the film of dried milk and the thin pellicle falls into a receptacle below. This is afterwards broken up and passed through a sieve, thus giving a uniform, finely granular powder.

The second method is used in the preparation of Trumilk,[106] in which the milk is first partially condensed in vacuo and then without being cooled is filtered and forced in a mist-like spray into a hot chamber through which air is circulating. The milk falls to the floor in a fine powder.

On account of their convenience in transportation and preservation, milk powders are coming into extensive use by bakers and confectioners, for whose purposes they are said to be fully equal, if not superior, to whole milk. They are also prepared for the use of travellers and campers in regions where fresh milk is not available, and as an ingredient of special foods for invalids and children. With regard to its bacterial content, dried milk compares very favorably with fresh milk, although it is not to be regarded as sterile.

Dried milks may be either full cream, half cream, or skimmed milks. In what follows unless otherwise specified full cream milk is referred to.

The concentration of the solids in dried milk is regarded roughly as eight times that in fluid milk, and the proportion in which the constituents are present is estimated to be as follows: [107]

Fat	25-28 per cent
Protein	25-28 " "
Lactose	34-40 " "
Ash	6-7 " "
Water	5-7 " "

Coutts [108] states that the albumin and globulin are at least partly coagulated. The caseinogen is not coagulated, but is probably altered in some way, the general concensus of opinion being that it is more digestible as found in dried milk than as in fresh milk. Any alteration which occurs in the fat or the lactose appears to be insignificant. The soluble salts of calcium are transformed, to some extent at least, into insoluble salts, but these are still available for nutritive purposes.[109]

[106] Merrell-Soule, Eng. Pat. 19,193, 1906.
[107] Lane-Claypon, p. 206.
[108] Coutts, l. c., p. 28.
[109] Daniels and Loughlin, J. Biol. Chem. 44, 396, 1920.

With regard to the vitamin content of dried milk, it might be expected that this product would not be inferior to evaporated milk in that respect, and might be superior, owing to the short period of exposure to heat. The experiments of Osborne and Mendel already referred to (p. 270) in which a diet of 60 per cent milk powder, 12 per cent starch, and 28 per cent lard was used, indicate that there is at least a fair amount of both *A* and *B* present, but when **Winfield**[110] attempted to keep rats on an exclusive diet of dried milk he found that while growth was normal for some weeks, often for as long as three months, it gradually became retarded and eventually ceased entirely, although the animals apparently remained in perfect health for the full period of the experiment, 16 months or more. As already pointed out (p. 271), it seems improbable that this failure in growth was due to lack of the recognized vitamins, since the fault was corrected by the addition of starch and lard, which are supposed to be free from vitamins. Moreover, although growth ceased there was no evidence of xerophthalmia, neuritis, or any of the other symptoms generally observed on a vitamin-poor diet. Use of fresh milk instead of milk powder did not induce renewed growth in the animals which had ceased to grow, but when a bread and milk ration was given growth recommenced.[111]

Winfield investigated 87 cases of infants on a dried milk diet and found that their growth curves closely resembled the average curves for breast-fed children, teething and walking began at normal ages, and there was no apparent liability to rickets or scurvy.

Naish, in a paper on " The Use of Dried Milk " given at a Conference on Infantile Mortality held in London in 1913,[112] states that: " We have in dried milk a food which contains the same substances as cow's milk and in the same proportions (except when humanized), which is adjustable to a wider range of infant which has obvious advantages of storage and distribution, and which appears to have no danger of the later nutritional disorders."

Sherman and his co-workers[112a] state that when dried milk took the place of fresh milk in their experiments with rats on a diet of bread and milk there was no evidence of any serious destruction of either *A* or *B*.

Several observers[113] have stated that the antiscorbutic in milk is

110 **Winfield,** Local Govt. Bd. Food Repts. 24, p. 146.

111 See also **Mattill** and **Conklin** and **Lane-Claypon,** quoted on p. 270.

112 " Pediatrics " 26, 247, 1914.

112a **Sherman, Rouse, Allen** and **Woods,** Proc. Soc. Exptl. Biol. Med. 17, 9, 1919.

113 **Pilgrim,** quoted by **Chick** and **Rhodes,** Lanc. 1918, ii, 774; **Hopkins,**

Fig. 21. The diet of this rat consisted of wheat flour 20, degerminated corn 10, cooked, dried potato 30, peas 10, navy beans 10, beets 5, turnips 5, and cooked, dried beefsteak 10 per cent.

This diet was derived from degerminated cereal products, legume seeds, tubers, edible roots, and meat (muscle tissue), but differed from that of Fig. 22 in not containing milk. It afforded wide variety, had an appropriate chemical composition in so far as analysis could show, was palatable. and included only natural food products of recognized wholesomeness, and from both animal and vegetable sources. Notwithstanding these facts the nutrition of the animals restricted to this food supply was very faulty. The photograph shows a rat at the age of 308 days, or at about the end of the first quarter of the normal span of life for a well-nourished animal of this species. It weighed but 137 gm. as compared with the one pictured in the following illustration whose weight was 245 gm. The pronounced stunting and very rapid aging under this diet emphasize the importance of including the foodstuffs designated as "protective foods." Milk is the most effective of these.

Fig. 22. The diet of this rat consisted of bolted wheat flour 20, degerminated corn-meal 10, potato 24, peas 8, navy beans 8, turnips 5, beets 5, beefsteak 10, and dry whole milk (Merrill-Soule) 10 per cent.

This diet was derived from the same foodstuffs as that employed above, and in closely similar amounts, and differed from it only in containing 10 per cent. of its dry substance as dry whole milk. The addition of this amount of milk corrected the faults in the remainder of the diet in a manner that is most surprising. The animal was 308 days old and weighed 245 gm. when photographed. It was, therefore, but little beyond one-fourth through the normal span of life for the species.

Figs. 21 and 22 supplied through the courtesy of Messrs. McCollum, Simmonds, Parsons and Journal of Biological Chemistry 38, 146, 1919.

reduced by the drying process. It is probable that there is considerable variation in the different dried milks on the market in respect to this factor, depending not only upon the temperature and time of heating, but also upon the length of time the product has been stored.

Barnes and **Hume** experimented with both guinea pigs and monkeys, and found that with both species a larger proportion of dried milk than of fresh was required to give protection against scurvy; in fact with guinea pigs they found it impossible to secure protection on an exclusive dried milk ration as the animals could not consume a sufficient amount of the food. The minimum protective ration of raw milk for monkeys was found to be 125 to 175 cc. daily, while it was necessary to give the equivalent of 250 to 300 cc. in dried milk freshly manufactured by the Just-Hatmaker process in order to secure the same degree of protection.

Hess and **Unger**[114] found that guinea pigs which had developed scurvy on a diet of hay, oats, and carrot water were cured by the addition of dried milk equivalent to 80 cc. of fresh milk to their diet. They concluded that dried milk[115] had lost none of its antiscorbutic power during the drying process, a conclusion in which they were confirmed by finding that infantile scurvy could be cured by giving dried milk of this variety. Barnes and Hume explain the discrepancy between these results and their own by the fact that Hess and Unger gave hay *ad lib.* in the basal ration, while they themselves gave only oats and bran. **Hart, Steenbock,** and **Smith**[116] have pointed out that a smaller amount of milk is sufficient to give protection on an oat-hay diet than on a rolled oats ration alone. These investigators could find no evidence of antiscorbutic in one brand of skimmed milk powder[117] which they investigated although their basal ration included hay *ad lib.*

On the other hand, **Davis**[118] confirms Hess and Unger, having maintained four guinea pigs for 400 days on a ration of hay and salt, with milk powder as the sole source of *C*. Although the ration was too concentrated for successful reproduction three young were reared on the same ration, and one of these gave birth to a litter of three of which two were reared. At the end of the experiment all nine were autopsied but no signs of scurvy could be detected.

Roy. Soc. Med. Sect. Ther. Pharm. Nov. 18, 1919, Lanc. 1919, ii, 979; **Barnes and Hume,** Bioch. J. 13, 306, 1919.

[114] **Hess** and **Unger,** J. Biol. Chem. 38, 293, 1919.

[115] Just-Hatmaker product.

[116] **Hart, Steenbock** and **Smith,** J. Biol. Chem. 38, 305, 1919.

[117] **Merrell-Soule** preparation.

[118] **Davis,** J. Home Ec. 12, 209, 1920.

Hart, Steenbock, and **Ellis**[119] conclude that milk powders vary in antiscorbutic properties. Apart from the initial quantity of *C* in the milk, due to variation in the feed of the cow, there may be considerable variation due to the method of manufacture, the spray processes in general being more destructive than the Just-Hatmaker process. They point out that this does not condemn spray process powders, but merely indicates their limitations when used as the sole source of nutrients in infant feeding. While it is probably safest to supply some potent source of *C* as supplement to all milk powders, there may be possible exceptions in the case of powders made by the Just-Hatmaker process from summer milks or even winter milks where the cow's ration has been made rich in antiscorbutic vitamin by proper selection of roots and tubers.

Jephcott and **Bacharach**[120] found the antiscorbutic value of summer and winter milks dried by the roller process to be about equal to one another and to those of the original raw milks. Milk neutralised with sodium bicarbonate before drying had a slightly inferior antiscorbutic value, and milk dried by the spray process was markedly deficient in antiscorbutic value. The dose of reconstituted dried milks required was 26 c.c. per 100 g. of body weight, the requirement of summer and winter milk being approximately the same.

119 **Hart, Steenbock,** and **Ellis,** J. Biol. Chem. 46, 309, 1921.
120 **Jephcott** and **Bacharach,** Biochem. J. 15, 129, 1921.

CHAPTER XVI

VITAMINS FOR THE BABY

It should be evident from what has already been said that the importance of the vitamins, especially in early life, can hardly be overemphasized. Apart from the specific diseases, scurvy, xerophthalmia, and perhaps rickets, which result from pronounced deficiency of these factors in the diet, there is a long list of less obvious disorders which might easily be ascribed to some other cause, or even escape notice altogether for a considerable time, but which are probably as inevitable accompaniments of a diet poor in vitamins as are the recognized "deficiency diseases" themselves.[1] **Schaeffer**[1a] believes the avitaminosis (vitamin deficiency) of young infants the most common of all and the most difficult to differentiate. Besides the marked decline and wasting of the tissues, frequently accompanied by xerophthalmia, which are regarded as indications of lack of *A*, diminished appetite, weakness, and lack of resistance to infection, anemia, and sometimes diarrhoea develop on diets which are inadequate in respect to this factor. Growth may be retarded, even where the deficiency is not so pronounced as to lead to actual wasting of the tissues. Derangement of the calcium metabolism is, according to **Mellanby**, a serious result, which may be manifested in the appearance of rickets and the production of defective teeth. Puppies which had been brought up on such a diet developed irregular and overlapping teeth, with the enamel partially lacking or very defective, the teeth in many cases being soft enough to be cut with a scalpel.

Deficiency of *B* leads to functional changes of almost all the organs of the body as well as to degeneration of the central nervous system. The glands of internal secretion and the digestive organs appear to suffer particularly. Acidosis frequently results from imperfect carbohydrate metabolism. **McCarrison** believes that many of the obscure diseases of children may be directly or indirectly due to lack of this vitamin. Moreover, deficiency of *B*, as of *A*, leads to lowered resistance and increased susceptibility to bacterial infection. Loss of appetite is one of the earliest symptoms and a very serious one, since

[1] See Ch. IX.

[1a] **Schaeffer,** Medecine (Paris), 1, 728, 1920.

it results in diminished intake of food and hence still greater deficiency of vitamins.

Lack of *C* is followed by the appearance of scurvy in a comparatively short time, but **Hess** in America, and **Barton**[2] have warned practitioners that the external symptoms of this disease indicated the later stages of deficiency, and that various ill-effects may have been developing slowly for some time. They therefore recommend the administration of antiscorbutic as a routine practice with all artificially fed infants, even where these are apparently healthy, a practice which has been advocated by **Pritchard**[3] also.

The calcium metabolism suffers from lack of *C* as well as from deficiency of *A*, resulting in abnormalities of the bony structure and of the teeth. It has been found possible to produce a condition in the teeth of guinea pigs closely resembling, if not identical with pyorrhea in man, by depriving them of adequate supply of *C*, and there is fairly convincing evidence for the belief that the defects of the teeth which are so prevalent in the present generation are due to insufficiency of vitamins in the food.

It is therefore of the utmost importance that the growing child be abundantly supplied with the necessary factors from early infancy.

The natural food for the young mammal is the milk of its mother. The variations in the milk of different species appear to be such as will correspond to the specific needs of the young of that species, and it may be assumed that the milk of a healthy mother on an ample and well-balanced diet will fully satisfy the physiological requirements of her offspring until it is ready for weaning. On the other hand, as has been pointed out already, if the diet of the mother is deficient in any respect, normal growth of the young can take place for a limited time only. Among the poorer classes in the Philippines, where the principal food is rice and fish, infantile beriberi is common among breast-fed children, and **Andrews** demonstrated that the milk of the mother of these children would produce antineuritic symptoms in puppies when given as the sole source of vitamin *B*. Scurvy, rickets, and pellagra have all been observed in breast-fed children, although such observations are rare compared with the number of cases among infants on artificial diets. **McCollum** and **Simmonds**[4] made an elaborate study of the effect produced upon the offspring by depriving lactating rats of definite dietary factors.[5] Where the mother's food

[2] **Barton, Lancet, 1919, ii, 348.**

[3] Pediatrics, 26, 300, 1913.

[4] **McCollum** and **Simmonds**, Am. J. Physiol. 46, 275, 1918; **McCollum**, "Newer Knowledge of Nutrition," N. Y. 1919, 117.

[5] See also **Drummond, Lanc. 1918, ii, 482.**

was entirely lacking in *A* growth took place at subnormal rate, indicating either that the young rats had a reserve store in their own tissues or that the mother was secreting a small amount of this vitamin from her own body fat. That the latter hypothesis is the correct one is indicated by the loss of weight by the mother. McCollum says: "It seems certain that the body fats of an animal which has been fed for some time on a diet rich in this substance *A*, will serve as a reserve supply of this dietary essential which the mother can secrete into the milk."

Experiments with diets deficient in *B* gave similar results, and McCollum concluded that neither *A* nor *B* is present in the optimum amount in the milk of the mother unless they are supplied in her food. Since, however, the young grew for a considerable time, although at slightly subnormal rate, when the mother was on a diet which would not sustain any growth in the young after weaning, it is evident that the mother can produce milk which is an improvement in respect to vitamin content, over the ration which she is herself receiving, either by concentrating in the milk the small store of vitamins which she gets, or by using up the reserve store in her own tissues. She is thus, as McCollum points out, a factor of safety for the nurslings. Similar observations were made in other experiments in which the diet was deficient in protein or mineral salts. While a certain amount of growth was possible, — in some cases a surprising amount, — both the young and the mother suffered. Commenting upon these experiments, McCollum remarks: [6] "The statement which one sees reiterated so frequently, that breast feeding of infants is superior to the best system of artificial feeding, needs to be qualified to some extent. There are, without question, in many parts of the world, large groups of people whose diets are of such a character that the quality of the milk produced by the lactating mother is not such as to make it a satisfactory food for their infants.[6a] It should be thoroughly appreciated that the human mother should have in her diet a liberal amount of milk in order to safeguard the health and well-being of her infant, and of leafy vegetables, which serve the two-fold function of a protective

[6] **McCollum,** "Newer Knowledge of Nutrition," p. 128.

[6a] From experiments conducted with rats, **Hartwell** (Biochem. J. 15, 140, 1920) has ascertained the effect of diet on mammary secretion. Provided the mother is given a good diet such as bread and whole milk, the baby rats at the end of the lactation period will weigh twice as much as when the mother is supplied with a poor diet, i. e. bread only. Extractives and protein tend to keep up the mother's weight. An excess of protein after a time brings about a cessation of the milk supply. Neither absence of fat nor an excess of carbohydrate appeared to have a disturbing effect. During the normal period of lactation, the mother can furnish vitamins *A*, *B* and *C*.

food and of greatly aiding intestinal elimination. That some mothers can induce a fair amount of growth in their infants while taking a faulty diet, cannot be denied, but that both mother and child suffer impairment as the result is beyond question. It is not enough that the diet shall furnish enough calories and enough protein, and shall afford variety and palatability. The peculiar dietary properties of the foodstuffs which enter into the diet are of paramount importance, and must be taken into consideration."

From his experiments on rats **Drummond** [7] has been led to believe that certain types of malnutrition common among the lower classes may be the result of diets more or less inadequate in regard to vitamin *A*, and particularly emphasizes the fact that young rats nursed by a mother whose diet was deficient in this respect never exhibit a normal resistance to disease.

When the mother's milk is not available for the infant, cow's milk in some form is almost invariably used as the substitute. This may be fed raw or after pasteurization, condensing, or drying and it is commonly modified in various ways in the attempt to approximate more closely the content of human milk. The effect of heat upon the various constituents of milk, including the vitamin content, has been fully discussed in Chapter XV.

In spite of the somewhat conflicting evidence it seems justifiable to conclude that a healthy child can develop normally on an exclusive diet of cow's milk, whether raw, pasteurized, dried, or condensed, provided a sufficient quantity can be fed. Even in raw milk, however, the supply of vitamins is near the minimum, and heating reduces this still further, especially if long continued. For this reason milk which has been pasteurized by the " flash " method, heating to boiling point or a little under for a minute and then cooling rapidly, is preferable to that which has been kept at comparatively low temperatures for longer periods, although this latter method is the one most generally used for pasteurization in the United States. For the same reason dried milk appears to be preferable to condensed milk for infant feeding, and the sweetened condensed milk is probably to be regarded as superior to the unsweetened " evaporated " variety which is frequently sterilized after condensation to improve its keeping qualities, although the high proportion of sugar in the sweetened product is probably a disadvantage.[8] Moreover, since aging appears to result in loss of *C* it is important to decrease the time between pasteurization and consumption as far as possible.

[7] **Drummond,** Lanc. 1918, ii, 482.

[8] This is denied by **Lassabliere.** See Lanc. 1918, ii, 54.

It seems to be the general impression that where whole milk in any form is the chief component of an infant's food there is little danger of deficiency of *A*. **Bloch's** experience in Copenhagen [9] indicated that a very small amount of this factor may be sufficient for successful nutrition. Milk fat appears to be the best source of this vitamin for infants; with older children eggs, and where necessary cod liver oil, may be used with advantage.

The feeding of children on separator milk to which vegetable fats have been added in order to bring this up to the fat standard of human milk is in general to be reprobated, owing to the deficiency of most vegetable fats in *A*. Linseed oil, which has been most commonly used for this purpose, is devoid of *A*, and most authorities agree that the use of this product as a substitute for butter-fat is likely to result in greatly diminished resistance to disease unless the necessary *A* is provided from some other source.[10] **Pritchard** and others report good results with an emulsion of soy bean oil, cotton seed oil, and suet, in proportions designed to resemble butter-fat. This is combined with sugar and dried separator milk to the standard of breast milk. Soy bean oil probably contains a small amount of *A*, and the beef suet is fairly rich in this factor. **Hampshire** and **Hawker** [11] recommend a somewhat similar emulsion of beef suet with a small quantity of olive or arachis oil. This product is known as "university cream" and is said to give good results.

Pritchard supplements such a food with fruit or turnip juice as an additional source of *C* and an ounce of meat and vegetable broth to increase the supply of *B*, along with small quantities of either yolk of egg or calcium glycerophosphates, which seem to be equally beneficial.

Daniels, Byfield, and **Loughlin** [12] have reported that addition of *B* as obtained from fruit or vegetable juice or from wheat embryo to a diet already furnishing an adequate number of calories invariably stimulated the growth of babies, while orange juice from which the *B* had been removed was of no effect. They also made use of a specially prepared vegetable soup as a milk diluent, with good results which they ascribe to the *B* present. They consider that the retarded growth of infants and young children is often the result of a deficiency of this vitamin in their food, and suggest that the relatively large food

[9] See p. 235.

[10] **Drummond,** Lanc. 1918, ii, 482; **Pritchard,** *Ib.*, 647.

[11] **Hampshire** and **Hawker,** Pharm. J. 103, 82, 1919.

[12] **Daniels, Byfield,** and **Loughlin,** Am. J. Dis. Child. 18, 546, 1919; 19, 349, 1920.

requirement of the artificially fed infant is connected with the poverty of diluted cow's milk in respect to this growth factor.

Eddy and **Roper**[13] have studied the effect of administering vitamin *B* in cases of infant malnutrition, and report excellent results in all cases (16 children). In one case they observed an average gain of 0.84 ounces per day over a period of 32 days, as compared with a gain of 0.47 ounces per day for 17 days preceding the use of the vitamin, the diet and food intake being the same through both periods. A three per cent increase in vitamin intake (as measured by the **Bachman** test[14]) produced marked stimulation. Eddy suggests that the pronounced effect of so small an addition indicates that extracted vitamin may be more readily utilized by the child than that present in the diet, and that the way in which the vitamin is held in the food may be an important factor in nutrition.

In discussing the proportion of *B* in milk, **Osborne** and **Mendel**[15] advise the use of a liberal amount of milk when this is depended on to supply any considerable proportion of this most necessary food factor. A case of this kind is that of infant feeding where it is customary to reinforce the supply of calories by diluting top milk and adding milk sugar. Under these circumstances the food contains a relatively smaller proportion of the water-soluble vitamin than does the original cow's milk. While milk thus modified may contain sufficient vitamin as long as the food intake is normal, if for any reason the child's appetite fails, the vitamin supply is reduced and endless dietary troubles may easily result. It is not improbable, they state, that a large part of the difficulties of artificially feeding babies is due to this cause, and that these can be obviated as successfully by securing an adequate supply of this indispensible constituent of a suitable diet as has been the case in feeding animals on artificial diets since we have learned how properly to provide this food factor.

Drummond[16] recommends modifying cow's milk with cream and whey only, and believes that if this is done there is little danger of deficiency of vitamins.

Bosworth has patented a method[17] for preparing a "reconstructed milk" of the approximate composition of human milk, by separating the fat (with the vitamin *A*) and casein from the serum of cow's milk together with practically all the calcium and phosphorus, but without any of the dissolved vitamins *B* and *C*, and finally re-combining the resulting serum with a portion of the fat and with protein and calcium and phosphorus in the proportions present in human milk.

[13] **Eddy** and **Roper**, Proc. Soc. Exp. Biol. Med. 14, 52, 1916; **Eddy**, Proc. Am. Soc. Biol. Chem. J. Biol. Chem. 41, xxxiv, 1920.

[14] **Bachman**, J. Biol. Chem. 39, 235, 1919.

[15] **Osborne** and **Mendel**, J. Biol. Chem. 34, 544, 1918.

[16] **Drummond**, Lanc. 1918, ii, 482.

[17] U. S. Patent 1,341,040.

As has already been pointed out various authorities recommend regular administration of additional antiscorbutic to all artificially fed infants. **Chick, Hume** and **Skelton**[18] and **Barnes** and **Hume**[19] place special emphasis on the necessity of giving an antiscorbutic ration to babies fed on heated or dried milk or any milk substitutes, on the ground that even in raw cow's milk this vitamin is near the minimum and that there is danger that any heat treatment may bring the amount down below the safety line.

Orange juice has long been used in infant feeding as a source of antiscorbutic. At University College Hospital, London, the minced orange, rind and pulp, is squeezed through a tincture press, and the juice collected. The essential oil from the rind acts as a preservative and the juice keeps for quite a long time. Doses of one teaspoonful in a little sugar water are given twice daily to infants under three months, and double that amount to babies over that age.[20] Where infantile scurvy has developed **Harden, Zilva** and **Still**[21] state that it may be successfully treated with lemon juice preparations from which the free citric acid has been removed by treatment with calcium carbonate and alcohol. When the irritant part was thus removed it was possible to give the equivalent of 6 to 12 lemons daily without any gastro-intestinal disturbance. **Hess**[22] recommends canned tomato unreservedly as the most serviceable antiscorbutic for artificially fed infants. It is well borne, comparatively inexpensive, and readily available. According to Hess it may be used in doses of one ounce per day in infant feeding. The juice of raw swedes (yellow turnips) is recommended by the English authorities[23] as the best substitute for orange juice where this is found too expensive for use. The antiscorbutic value of these vegetables is high[24] and they have the merit of being abundant and cheap. The clean cut surface of the raw turnip is grated on an ordinary kitchen grater and the pulp folded in a small piece of muslin and squeezed with the fingers, till the juice runs out. **Barlow**[25] and **Hess** and **Fish**[26] have reported favorably upon the use of potatoes as an antiscorbutic. This may be made into a cream by shaking up well cooked potato in water, and fed as a diluent of cow's milk.

[18] **Chick, Hume** and **Skelton,** Lanc. 1918, ii, 1.
[19] **Barnes** and **Hume,** Bioch. J. 13, 306, 1919.
[20] **Barton,** Lanc 1919, ii, 348.
[21] **Harden, Zilva** and **Still,** Lanc. 1919, p. 17.
[22] **Hess,** N. Y. State Jour. of Med. 20, 209, 1920.
[23] Med. Res. Com. Rep. 38, p. 80.
[24] **Chick** and **Rhodes,** Lanc. 1918, ii, 774.
[25] **Barlow,** Brit. Med. J. 1894.
[26] **Hess** and **Fish,** Am. J. Dis. Child. 8, 385, 1914.

It should be noted that tomato and turnip juice are good sources of *A* and *B* as well as of *C*, so serve a triple purpose. Orange juice is about equivalent to milk as a source of *B* and may possibly contain *A* as well, but in very small quantities.

The fact that the proportion of children developing "deficiency diseases" on artificial diets which are unquestionably low in vitamins is small has led to the suggestion [27] that the infant starts life with a reserve supply of these factors sufficient to maintain it until its milk diet can be supplemented with other foods. Even if this is the case, however, it may be questioned whether this reserve alone is sufficient for optimum development. Although no direct experimental evidence is available on this point the low mortality among breast-fed, as compared with artificially fed, infants during the first year of life suggests that their resistance may be increased by a higher vitamin content in human than in cow's milk. In the later stages of life, when the milk diet begins to be supplemented by other foods, the difference between the two classes tends to disappear.

Norman [28] states that he considers the superiority of breast-fed children as compared with bottle-fed ones is not so well marked at school age as one would be led to expect. It is during the early years of life, prior to school age, that the great advantage of breast-feeding over bottle-feeding is so apparent. The bottle-fed children of the poor that survive, appear to overtake their disadvantages, and by the time we find them in school, show no inferiority physically to their breast-fed fellows.

The variation in development of infants on identical diets may very possibly be due to variation in the reserve supply of vitamins which they are provided at birth, and this in turn may be at least partly dependent upon the vitamin content of the food of the mother during pregnancy.

[27] **Gibson and Concepcion**, Phil. J. Sc. B. 1916, ii, 119; **Hopkins**, Lanc. 1919, ii, 979.

[28] Quoted by Lane-Claypon, "Milk and its Hygienic Relations," p. 312.

CHAPTER XVII

OLD FOODS THROUGH NEW EYES: WHAT TO EAT FROM THE VITAMIN STANDPOINT

THE question which follows naturally from a study of the vitamins and their importance in the maintenance of health is how much vitamin-containing food is required in the daily ration? Unfortunately that is a question to which it is impossible to give a categorical answer in the present state of our knowledge. It is generally assumed that one pint of unpasteurized milk a day supplies at least the minimum amount of all three vitamins for a growing child, but it is far from certain that this is the optimum amount. On the contrary experiments already referred to (p. 296) would seem to indicate that a child may be highly benefited by increasing the vitamin content of the food above the customary amount. Still less is known as to the quantitative requirement of adults. *A* is apparently dispensable after growth ceases, but it is not known with certainty whether the system is actually maintained in perfect health and efficiency over long periods of time on a diet deficient in this factor. It has been shown (p. 179) that the amount of *B* required may depend upon the character of the diet in respect to protein, fat, and carbohydrate. It is still uncertain what relation exists between muscular activity and vitamin requirement. It must be continually borne in mind that absence of pronounced disease is not a satisfactory criterion of health. Only when every organ in the body is in a condition of highest efficiency should the individual be regarded as satisfactorily nourished. Malnutrition is probably a much more common condition than we recognize, and we can hardly decide at this stage how far a deficiency in vitamins may be responsible for such condition. On the other hand, so far as we know there is no possibility of overloading the system with vitamins, and it would appear the part of wisdom to so regulate our diet as to ensure the maximum consumption of the vitamin-rich foods. While this is most important in early life it should not be disregarded at any period.

The vitamin content of milk has already been discussed fully. Cream and top-milk may be used as a source of *A*, and are eminently

satisfactory where expense need not be considered too carefully. Skimmed milk retains the antineuritic and antiscorbutic vitamins, but it must be used very liberally if it is to form the sole source of these vitamins. Curran reports that an allowance of one pint of milk per person per day was not sufficient to prevent an outbreak of scurvy in a Dublin work-house when the usual allowance of potatoes was reduced by a potato famine. On the other hand a diet which is poor but not entirely deficient in vitamins can be very conveniently supplemented by the addition of one or two glasses of milk per day.

Butter is a valuable source of *A* as well as an appetizing form of fat, but unfortunately under present day conditions it is too costly to be used as freely as might be desired. The increasing cost of good dairy butter has led to the substitution of oleomargarine on many tables. Oleomargarine, or margarine as it is called in Europe, is a name applied to any butter substitute made by churning fats other than milk fat with milk. A great variety of fats is used for this purpose, some of them being obtained from animal and others from vegetable sources. Of the animal fats those most commonly used are "oleo oil" and "neutral lard." In the preparation of "oleo oil" beef fat is rendered, clarified, cooled to solidification and separated by hydraulic pressure into the lower-melting oleo oil and the higher-melting oleo-stearine, the latter being used chiefly in the preparation of cooking fats. "Neutral lard" is a carefully prepared lard which is free from taste and odor and is used in the preparation of high-grade oleomargarines. Among the principal vegetable fats used are coconut oil, cotton-seed oil, arachis, or peanut oil, linseed oil, corn oil, and soy (or soya) bean oil. The use of hydrogenated fats prepared by treating liquid oils with hydrogen in presence of a catalytic agent, has increased rapidly within the last few years. By this means inedible oils such as whale and other fish oils, can be transformed into tasteless, odorless fats of almost any desired melting point. Besides fish oils, soy bean, cotton-seed, rape, peanut, sesame, linseed, and sunflower oils are hardened and used in the manufacture of oleomargarines and lard substitutes. A fat or mixture of fats, of the proper consistency having been obtained, it is churned with milk which has been soured by a pure culture of lactic acid bacilli to give it a pleasant flavor. Butter or cream are sometimes added in varying proportions for the production of the better grades. In some of the European countries the amount of butter-fat introduced is restricted by law, but in the United States any desired amount may be used.

All the experimental evidence goes to show that the fats of the oleo-

margarines are as digestible and as readily assimilated as butter-fat. The only nutritive superiority of butter lies in its content of the vitamin *A*. Oleomargarines vary considerably in respect to this factor, according to the fat used in their manufacture. The vegetable fats in general are poor in *A* and an oleomargarine prepared entirely from such fats will contain only insignificant traces of *A* from the milk used in churning. Oleo oil is fairly rich in *A*, although not equal to butter-fat. Animal experimentation has demonstrated that oleomargarines prepared from beef fat will not only serve as adequate source of *A* for the maintenance of growing animals in normal condition but will restore animals which have suffered decline from deprivation of *A*. It should, however, be used somewhat more liberally than butter if it is to form the sole source of this vitamin. Lard probably is devoid of vitamin, so that the use of neutral lard does not improve a margarine in this respect. The fish oils are rich in *A*, but unfortunately this factor is destroyed in the process of hydrogenation. It may be noted, however, that this does not constitute an objection when the hydrogenated fat is to be used as a lard substitute since, as noted above, the lard itself is presumably vitamin-free.

Since it has been found possible to obtain potent concentrated extracts of the *A* vitamin from the grasses, there seems no reason why it should not be possible to enrich a vitamin-poor fat with such extracts and thus remedy the only nutritional defect of the butter substitute. Meantime it is not necessary to avoid the use of the margarines on this ground, provided a sufficient amount of *A* is supplied from other sources. The enemies of oleomargarine have been criticized for making a seemingly unwarranted trade use of data on the very limited content of *A* in margarines. It is neither reasonable nor fair to the very important and essential vegetable or nut-butter industry to condemn these products on the ground that vitamins are not present. The amount of animal fats produced is hopelessly inadequate to supply the world's requirements. Vegetable fats necessarily must form a part of the diet of mankind. We deplore the attempts to play upon the defect for trade purposes. Instead, endeavors should be put forth to improve the body-building values of vegetable fats by the addition of vitamin concentrates to these fats.

Whole milk cheese is fairly rich in *A* and may contain a small amount of *B* as well although it cannot be regarded as a satisfactory source of this vitamin. Skim-milk cheese is practically vitamin-free

Eggs are rich sources of *A* and *B*. The fat of egg-yolk is as efficient as butter-fat in growth-promoting power. Twenty grams of dried

whole egg (about half an egg) was sufficient to cure a polyneuritic pigeon. There is no evidence, however, that they possess any antiscorbutic power. A diet of milk and eggs, whether raw or cooked, would therefore supply an abundance of the *A* and *B* vitamin but can with advantage be supplemented with respect to *C*.[1]

Cooked meats add little to the vitamin content of the diet. Some *A* may be present in the fats, and small quantities of *B* and *C* may be obtained from the lean muscle tissue, but in general meat and fish may be disregarded as a source of vitamins unless used more extensively than would be advantageous for the average individual in civilized life. The glandular organs are exceptions to this, however, the liver and kidney being rich both in *A* and *B*. The pancreas (sweetbreads) and brain also contain considerable amounts of *B*. **McCollum**[1a] states that it is possible to select foods so as to secure a fairly satisfactory diet entirely derived from animal tissues, provided the ration is not limited to muscle tissue. When blood, liver, kidney, and other glandular tissues are selected, together with a certain amount of bone substance, the food supply is sufficiently good to provide for normal development. Young animals cannot grow or remain long in health, however, when restricted to muscle tissue as the sole food. With reference to this it is pointed out in an editorial in the American Medical Journal[2] that the avoidance of glandular organs on account of their high purin content is probably unnecessary. Over-eating of glandular tissue is likely to be unwholesome. " But assuming moderation of diet on the part of a healthy man in a regimen in which liver and its analogues are consumed as adjuvants rather than fundamentals in the ration, it is likely that they may often exercise a wholesome and even corrective influence in supplying food factors which the cereals, roots, tubers, and even muscle tissue lack."

[1] The value of vitamins contained in ice cream is discussed by Jaffa. (Chem. Abs. 1921, 2516).

Concerning Farmers' Bulletin 1207 issued by the States Relation Service, Department of Agriculture, the American Food Journal (November, 1921, 24) notes that the discussion of vitamins in this bulletin marks the complete acceptance of these discoveries by the States Relations Service. Referring to the vitamin *A*, found especially in milk fat, the bulletin says: " Vitamin *A* is of special importance for two reasons: one is that without it children cannot grow and develop normally, even though their food is otherwise sufficient for their needs; the second is that vitamin *A* is found in such foods as milk, egg yolk, green leaf vegetables, fats surrounding the vital organs, organs of animals, to a less extent in meat, and perhaps in certain fruits and in few so abundant as in milk. It appears to go with the milk fat and so is found in whole milk, cream and butter."

[1a] **McCollum**, Proc. Inst. Med. Chic. 3, 13, 1920.

[2] Am. Med. J. 75, 1206, 1920.

Since milk and eggs are used on the whole rather sparingly in the diet of the average adult and meat is not a very abundant source of vitamins, the vegetable foods must form the main source of supply of these factors. It is unfortunate that flour, which makes up so large a proportion of the daily ration, should have been robbed of its vitamin content in the milling process, but the refined product of the modern mills is useless as a source of vitamins. Whole wheat bread furnishes both *A* and *B*, and the combination of whole wheat bread and butter, milk, and fruit is an admirable one as regards vitamins.

Apart from the cereal grains the seeds commonly used as foods contain no quantity of *A*. Peas and beans, fresh or dried, furnish a fair amount of *B*. *C* appears to be lacking, except on germination.

Tubers and roots differ surprisingly in their content of vitamins, so much so that no generalization can be made. The white potato, the most extensively used root vegetable, is relatively poor in both *A* and *B*, but may serve as an effective antiscorbutic if supplied liberally. Sweet potatoes have a higher concentration of *A* than white potatoes. The common yellow turnip (swede or rutabaga) is a good source of all three vitamins, although the antiscorbutic is somewhat decreased by the necessary cooking. Carrots contain about equal amounts of *A* and *B*, but are much poorer in antiscorbutic than the rutabaga. Beet-root is comparatively poor in all three vitamins.

The leafy vegetables, spinach, cabbage, and lettuce, are exceptionally rich in *A*, spinach being the richest and lettuce the poorest of the three, and are also excellent sources of *B* and *C*. The same statement may be made of the tomato. These four vegetables are commonly regarded as luxuries rather than necessities, and are generally used chiefly to give variety to the menu. Our recently acquired knowledge of the vitamins indicates, however, that they should play a much more prominent part in our diets. **McCollum** has placed a great deal of emphasis on the value of the leafy vegetables which he classes along with milk as protective foods. He points out that while these vegetables might at first sight appear to be uneconomical foods, owing to their low content of carbohydrate, fat, and protein and high content of water, they have nevertheless a peculiar value owing to their high vitamin and mineral content and should be used as liberally as possible.

The same argument may be advanced for the generous use of fruits. These are particularly valuable for their antiscorbutic properties, but most common fruits contain a small amount of *B*. Bananas contain some *A*, but little or no *B*.

Common varieties of edible nuts appear to be of little value as a

source of *A*, although walnuts are said to contain some of this factor. On the other hand, nuts in general appear to contain considerable quantities of *B*. Information is lacking as to their antiscorbutic value.

Suggestions by **Delf** (Science Progress, **15**, 601, 1921) are useful to dietitians when considering foods from the vitamin standpoint. If butter, milk or animal fats are scarce the quantity of lightly-cooked green vegetables and also carrots, or raw vegetables served as salads, should be increased. Fat fish such as herring, mackerel and salmon, if available, are recommended. Cod liver oil is especially useful for children. Germinated pulses or cereals may replace fruits and vegetables when the latter are scarce. One-half pound of vegetables supplies the daily needs of a man doing full work. The juice of an orange or lemon, or a fairly large fresh tomato daily affords sufficient vitamin *C* for an adult.

Yeast has been highly recommended as a source of vitamin *B*, but in order to be efficient it must be used liberally, the amount ordinarily used in bread-making being insignificant. **Hawk** [2a] recommends the use of flour containing 5 per cent of yeast powder for bread making, stating that the product is palatable and much more nutritious than ordinary bread, both the vitamin *B* and the protein of the white flour being supplemented by the yeast. **Renshaw** [2b] has obtained some evidence indicating that yeast is effective in increasing the utilization of other foods.[2c] Excellent results have followed the therapeutic use of yeast in disorders of the blood, and so far no deleterious effects have been observed from its consumption on a liberal scale. On the other hand, where the diet can easily be regulated at will it is a simple matter to arrange palatable combinations of the ordinary foods which will supply an abundance of vitamins of all three classes without the addition of yeast.

Evidence is not yet complete as to the amount of destruction undergone by the three vitamins on heating. Certainly there is strong probability that more or less deterioration takes place at high temperature, and consequently that larger quantities of cooked foods will be

[2a] **Hawk, Smith and Bergeim,** Am. J. Physiol. **56**, 33, 1921.

[2b] **Renshaw,** Am. Naturalist **45**, 73, 1921.

[2c] White mice were fed on a basal diet with varying amounts of yeast. The diets prepared were: *(a)* casein 17.5%, starch 49.5%, lard 18%, butter 9%, salts 5%, yeast 1%; *(b)* casein 16.5%, starch 48.5%, lard 18%, butter 9%, salts 5%, yeast 3%; *(c)* casein 15.5%, starch 47.5%, lard 18%, butter 9%, salts 5%, yeast 5%. On diet *(a)* in unlimited amount the net loss of 12 mice in 13 days was 2.93 g. They were then fed diets *(b)* or *(c)* in limited amounts when the net gain was 14.38 g. in 16 days.

required to supply the necessary factors than of the same foods in the raw state. As the antiscorbutic is the most sensitive of the vitamins to temperature a diet made up entirely of cooked foods is likely to be deficient in this respect, a danger which is, however, easily avoided by the addition of salads and raw fruits to the menu.

As has been shown the destruction increases rapidly with temperatures above 100° C. Consequently baked or fried foods may be expected to be poorer in vitamins than boiled foods. On the other hand, exposure to a low temperature for a long period has been found more destructive to the antiscorbutic factor than a higher temperature for a shorter time. It is therefore desirable that foods which are relied upon to furnish the vitamin *C* in the diet should be cooled as rapidly as possible, if they must be cooked at all. In several cases outbreaks of scurvy in military camps which occurred in spite of a daily ration of fresh vegetables have been traced to the fact that these vegetables had been subjected to long slow cooking or stewing which entirely destroyed their antiscorbutic value. **Chick** and **Dalyell**[3] have reported an outbreak of scurvy which affected 40 children out of 64, between 6 and 14 years of age, who were under treatment for tuberculosis. Fresh vegetables were supplied regularly in generous amount, but it is assumed that the long cooking to which they were subjected had destroyed the greater part of the vitamin present. They suggest[4] that chopped cabbage and tomato be added to soup a short time before serving, in order to reduce the heating of these vegetables to a minimum. Or, when fresh fruit or vegetables are difficult to obtain, turnips and carrots might be grated and the juice set aside, adding the grated vegetable to the soup to cook for as long as necessary, but pouring in the juice just before the soup is served. Cabbage and cauliflower should be steamed instead of boiled, in order to retain the vitamin which would otherwise be lost in the water.[4a]

The Medical Research Commission[5] has pointed out in this connection that food cooked in a fireless cooker will lose much more of its antiscorbutic factor than it would if cooked in the ordinary way. This is not necessarily an argument against the use of fireless cookers, however, nor against the eating of baked or roasted foods, but points

[3] **Chick** and **Dalyell**, Brit. Med. J. 1920, 2, 546.

[4] **Chick** and **Dalyell**, Ztsch. f. Kinderh. 26, 257, 1920.

[4a] **Delf** deplores the practice of over-cooking or "keeping hot" so frequently employed by the housewife in preparing fruits and vegetables. These should be heated only to an extent sufficient to sterilize and soften the tissues. (Science Progress 15, 601, 1921).

[5] Report 38.

to the necessity of supplementing such food with some other source of *C*.

The stability of the vitamins may vary considerably according to the source in which they occur, and, on the other hand, different foods differ as to the amount of heat necessary to ensure their preservation. It is at present impossible therefore to make an entirely satisfactory general statement as to the vitamin content of canned and preserved foods. According to **Steenbock** and **Boutwell**, heating under pressure for three hours does not cause any noticeable destruction of *A* in corn, chard, carrots, sweet potatoes or squash. **McCollum** asserts that there is not the slightest danger of destroying this vitamin in any vegetables or fruits by the ordinary canning process. **Delf**, on the other hand, reports a serious amount of destruction of the *A* vitamin in cabbage after heating to 130° for two hours.

Chick and **Hume** have stated emphatically that all canned and sterilized foods are likely to be deficient in *B*, and although a few observations are not in accord with this, it is wiser not to depend on such foods for our supply of antineuritic.

It is the general concensus of opinion that the antiscorbutic factor is likely to be seriously depleted by heating for long periods or to a high temperature, and that canned goods cannot be depended upon to furnish this vitamin. Tomatoes are a marked exception to the general rule, however, canned tomatoes being still very satisfactory sources of the three vitamins.

There is very little evidence as to the effect of drying on vitamin *A*, but it is probable that this factor will not be seriously injured by the drying process. *B* appears to be little if any affected by drying at moderate temperatures. There is considerable variation in the stability of *C* in different foods, and a few dried foods retain some antiscorbutic power, but it is usually considerably reduced, the deterioration increasing on storage. The majority of fruit juices are rather exceptional in this respect, retaining their potency for months if dried under suitable conditions. For the most part, then, dried foods may be expected to supply practically the same amount of *B*, and possibly as much *A* as the same foods when fresh, but are not to be relied upon for antiscorbutic. They may be used to give variety and palatability and have a certain amount of nutritive value, but they cannot take the place of fresh vegetables and fruits.

From the point of view of economy it is doubtful whether the preservation of vegetables by canning and drying is generally profitable. It is possible to obtain all the vitamins required from those vegetables which keep without deterioration throughout the winter months in

their natural state, such as potatoes, turnips, carrots, parsnips, cabbage, and celery, and the winter fruits, apples, oranges, lemons, and grape fruit. By restricting ourselves to these in the winter and eating the more perishable products only in summer when they can be obtained young and fresh from the garden much fuel and labor would be saved without the sacrifice of any nutritional advantage. As already mentioned, however, tomatoes occupy an exceptional position, as they are preserved much more easily than the majority of vegetables and retain their vitamin content practically unchanged.

The probable future development of vitamin concentrates of uniform reliable quality may make possible the enrichment of our favorite foods by the addition of vitamins. Those favoring vegetable fats can have nut-butter vitaminized. Some persons dislike milk and others refuse butter. Vitamin *A* could be administered through the addition of a concentrate to soups or other foods. The Jap rejects rice which is not white. If " off color " it does not satisfy his expectations. But when his ankles swell and pains are felt in the calves of the legs, due to incipient beriberi, he has learned to drop rice from the diet temporarily and to eat whole wheat. He does this most reluctantly and a means for cheaply adding vitamins to polished rice should be popular in Japan.

CHAPTER XVIII

THE USE OF THE VITAMINS IN CLINICAL MEDICINE

There is comparatively little material available at present on the use of vitamins in clinical medicine. The whole subject is too new and too little understood to have had widespread application as yet. Nevertheless, certain extremely interesting and suggestive articles have appeared, in which those best fitted to speak with authority on the question have urged upon physicians in general the necessity for greater attention to these factors.[1]

It cannot be too strongly emphasized that the so-called " deficiency diseases " are not the only, or the most important, results of deficiency of vitamins. In the Harvey Society Lecture, 1921, **Hess**[2] urges physicians to realize that a lack of these essential food factors generally does not bring about typical pathological states, but obscure alterations of nutrition; ill-defined functional disabilities, which cannot be characterized or even recognized as disease and that it is such incomplete, larval forms of the deficiency disorders to which physicians will have to address themselves. Further, Hess argues as to the probability that every organ or system in the body may be affected by faulty nutrition, so that the deficiency diseases must engage the attention of every physician, whatever his particular interest or specialty. For example, involvement of the eyes may lead to impaired vision or night blindness, or, on the other hand, neuritis, cardiac enlargement, disturbances of the circulatory system, atrophic disorders of the skin, nails or hair, caries of the teeth, or unaccountable lack of appetite and constipation may each in turn be the earliest symptom. A more careful inquiry into the dietary of patients will result in bringing to light many cases in which vague and ill-defined symptoms can be remedied simply by rendering the diet adequate. The chief clinical importance of disorders of nutrition seems to be associated with the fact that they bring about an abnormal condition of the tissues

[1] **Hopkins, Barr,** et al. British Med. J. 1916, 2, 147; **McCarrison,** Ind. J. Med. Res. 6, 275, 1919; **Chick and Dalyell,** British Med. J. 1920, 2, 546; **Hess,** J. Am. Med. Assn. 76, 693, 1921.

The growth of tumors may be stimulated by accessory factors. From their experiments on yeast, **Suguira and Benedict** suggest that the favorable effect of radium in such cases may be due to the partial destruction of accessory substances (see **Zunz,** Scalpel, June 19, 1920; Physiol. Abstracts 5, 556).

[2] **Hess,** J. Am. Med. Assn. 76, 693, 1921.

which renders them more susceptible to the invasion of bacteria or their products. This relationship was exemplified in 1913, when, as the result of a dietary of pasteurized milk, latent scurvy developed among a group of infants under observation by Hess. This " scorbutic taint " was followed by a widespread grip infection with hemorrhagic skin manifestations, which disappeared on the administration of orange juice. For some years Hess was uncertain how to interpret this peculiar clinical picture, whether to regard it as due to scurvy or to infection. As the result of subsequent experience he realized that it was due to both causes, the result of a primary nutritional disturbance and a secondary bacterial invasion. Another illustration of the inter-relationship of disordered nutrition and infection was furnished by the frequent coincidence of nasal diphtheria and latent or subacute scurvy. This concurrence is so suggestive that when a large number of cases of nasal diphtheria develops, suspicion should be aroused that the infection is implanted on tissues rendered susceptible by scorbutic or other nutritional disorders. This " nutritional-infectious " aspect has been convincingly illustrated on a large scale among the peoples of the Central Empires, who during the many years of the war suffered from various forms of malnutrition. The general impairment of health was most strikingly manifested both in adults and in children by the great spread of tuberculosis and its increased mortality. **Davidsohn** [3] has reported that in Berlin there was a marked increase in infection with tubercle bacilli in children under the age of five years, and they had been infected earlier in life than formerly; whereas in 1913, 30 per cent gave positive reaction at four and one-half years, in 1919 this percentage was reached at two and one-half years.

Reference has already been made to the observations of **McCarrison** and others on the effect of vitamin deficiency on the various organs [4] and are in themselves highly suggestive. McCarrison states that his clinical experience leads him to believe that there are many minor maladies associated with deficiency of the accessory food substances in the diet of children especially, or with incomplete assimilation of these factors, and remarks that the laboratory experience gained in animal experimentation with vitamins had been of no small measure of assistance in dealing with cases of bilious vomiting, acidosis, mucous disease, and other metabolic disorders of childhood which were referred to him.

The most common dietary faults among children of the well-to-do are considered by **McCarrison** [4a] to be a deficiency of vitamins and

[3] **Davidsohn,** Ztsch. f. Kinderh. 21, 349, 1919.

[4] Chap. IX.

[4a] Lancet 1921, I., 348.

an excessive amount of carbohydrates with low salt content, especially calcium, when milk is overheated and vegetables are scanty. The effects of such faulty foods as observed experimentally on monkeys have been: Congestion and hemorrhage in the wall of the stomach and intestine; atrophy of the myenteron; degenerative changes in Auerbach's plexus; dilations in various parts of the tract; increased tendency to intussusception; inflammations of the colon; bacterial infection; depressed functional activity of the intestinal glands. Continued subsistence on such diets may lead to intestinal stasis particularly in its effects on the neuromuscular control of the bowel. Secondarily there may arise disturbances in the endocrine organs, particularly the adrenal.

In the early days of vitamin research **Huessy** reported[5] that orypan, a remedy prepared from rice husks,[6] of which the principal constituent is probably vitamin *B*, was used with good results in the treatment of weakness and inanition of women.

Hawk, Knowles, Rehfuss, and **Clarke**[7] have published the results of an investigation into the therapeutic value of baker's yeast (Fleischman's), another substance which most probably owes its potency almost entirely to its content of vitamin *B*. Their results are summed up as follows:

Condition	Number of Cases	Improved or Cured
Furunculosis	17	16
Acne vulgaris	17	17
Acne rosacea	8	8
Constipation	10	9
Gastro-intestinal catarrh	3	3
Intestinal intoxication	1	1
Eczemas	5	0
Arthritis deformans	1	1
Psoriasis	4	1
Erythema and urticaria	1	1
Bronchitis	2	2
Urethritis	2	2
Pruritus	1	1
Folliculitis	1	1
Conjunctivitis	1	1
Duodenal ulcer	1	1
Swollen glands	1	1
Totals	76	66

[5] **Huessy,** Munch. Med. Woch. 61, 981, 1914.

[6] See p. 123.

[7] **Hawk, Knowles, Rehfuss,** and **Clarke,** J. Am. Med. Assn. 69, Oct. 13, 1917.

These investigators add that in many of the cases which came under their observation, the yeast treatment caused an improvement in the general physical condition of the patient quite unassociated with the improvement of the symptoms associated with the particular disease in question.[8]

[8] Certain experiments on fermentation by **Schweizer** (Mitt. Lebensm. Hyg. 11, 200, 1920) according to his interpretation do not confirm the existence of vitamins in yeast.

CHAPTER XIX

VITAMINS AND THE LOWER ORGANISMS

IN 1901 **Wildiers**[1] made the statement that yeast will not grow in a nutrient solution containing ammonium salts as the sole source of nitrogen if the amount of the inoculating material is small. If the amount of the latter is large, fermentation soon follows, presumably because an essential nutrient material is introduced through the death and autolysis of some of the cells. To this hypothetical nutrient Wildier gave the name "bios." As the knowledge of vitamins developed, a general resemblance between the behavior of "bios" and vitamin *B* became apparent, so much so that the Belgian chemists who are studying "bios" have adopted many of the methods devised for the study of vitamin *B*,[2] while, on the other hand, several American workers have endeavored to use the growth of the yeast plant in a suitable medium as a quantitative test for the presence of vitamin *B*. The methods employed and the quantitative results obtained are discussed elsewhere. (See Ch. II.)

Leaving aside the question of the identity of "bios" with any of the known vitamins, a brief summary of the work done on the effect of vitamins, or unidentified vitamin-like factors, on yeasts, moulds, and bacteria follows.

[1] **Wildiers**, La Cellule, 18, 313, 1901. See also **Liebig**, Ann. Chem. 153, 1, 137, 1870; **Pasteur**, Ann. Chem. phys. 25, 145, 1872; **Mayer**, Ann. Physik. 142, Ser. 1, 293, 1871; **Nägeli**, Chem. Zentr. 12, 45, 188; **Amand**, La Cell. 21, 329, 1904; **Devloo**, *Ib.* 23, 361, 1906; **Henry**, Ann. brass. et dist. 1902, 129; Woch. brau. 19, 325, 1902.

[2] **Ide** observes that a distinction should be made between two kinds of proliferation of yeast, one very slow without "bios" and one fast with "bios." To achieve rapid growth in which proliferation is increased 30 times a special "biosine," specific in its influence on the growth of yeast, is asserted by Ide to be necessary for he considers that none of the known organic substances described up to the present time has any comparable influence on yeast. For rapid growth, the yeast needs a large portion of its nitrogenous food in the form of biosine. As yet, no difference has been observed in the chemical properties of the water-soluble *B* of **Myers** and **Voegtlin** and biosine. But it cannot be stated yet with certainty that water-soluble *B* vitamin and "bios" are the same substance.

Bachmann,[3] **Williams,**[4] **Swoboda,**[5] **Eddy** and **Stevenson**[6] and **Funk** and **Dubin**[7] have all believed that the vitamin requirement of yeast is so definite that it may be made the basis of a quantitative test for the presence of vitamin *B*, although the last-named authors have now come to the conclusion that the vitamin in question is not *B* but a new vitamin which they call *D*,[8] and Bachmann pointed out that anaerobic yeast could apparently develop successfully in a vitamin-free medium.

Emmett and **Stockholm,**[9] **Whipple**[10] and **Eddy, Heft, Stevenson,** and **Johnson**[11] agreed that vitamin preparations have a highly stimulating effect on the growth of yeast,[12] but doubted the applicability of the method for quantitative determinations, while **Souza** and **McCollum,**[13] **MacDonald** and **McCollum,**[14] **Fulmer, Nelson** and **Sherwood**[15] deny that a supply of vitamins is essential for the growth of the yeast plant. As in so many other branches of vitamin research the results obtained in different laboratories seem directly contradictory, but it is probable that with further knowledge a compromise is already suggested by the work of Funk and Dubin referred to above, in which they believe they have secured conclusive evidence that the growth stimulant required by yeast is not *B* but another vitamin with many properties similar to those of *B*, but quite distinct from it in others. (See p. 150 and ff.)

That water soluble *B* is not an essential constituent of the medium for the growth of yeast but yeast is capable of synthesizing that vitamin is indicated by the observations of **Nelson, Fulmer** and **Cessna.**[16] For growing the yeast a medium was used of the following compositions:

[3] **Bachmann,** J. Biol. Chem. 39, 235, 1919.
[4] **Williams,** *Ib.* 38, 465, 1919; 42, 259, 1920; 46, 113, 1921.
[5] **Swoboda,** *Ib.* 44, 531, 1920.
[6] **Eddy** and **Stevenson,** Proc. Soc. Exp. Biol. Med. 17, 52, 1919.
[7] **Funk** and **Dubin,** J. Biol. Chem. 44, 487, 1920; 48, 437, 1921.
[8] See also **Emmett** and **Stockholm,** J. Biol. Chem. 43, 287, 1920.
[9] *Loc. cit.*
[10] **Whipple,** J. Biol. Chem. 44, 175, 1920.
[11] **Eddy, Heft, Stevenson** and **Johnson,** *Ib.* 47, 249, 1921.
[12] See also **Euler** and **Pettersson,** Z. physiol. Chem. 114, 4, 1921.
[13] **Souza** and **McCollum,** J. Biol. Chem. 44, 113, 1920.
[14] **MacDonald** and **McCollum,** *Ib.* 45, 307, 1920–21; 46, 525, 1921.
[15] **Fulmer, Nelson** and **Sherwood,** J. Am. Chem. Soc. 43, 186, 191, 1921.
[16] J. Biol. Chem. 46, 77, 1921.

Potassium Phosphate	0.1 g.
Calcium Carbonate	0.04 g.
Calcium Chloride	0.1 g.
Ammonium Chloride	0.188 g.
Sucrose	10. g.
Distilled water	100 c.c.

The yeast was plated from a Fleischmann yeast cake. The yeast was grown continuously in the above medium for a year. It was grown in 50 cc. portions of the medium, 1 cc. of the culture being transferred every other day to 50 cc. of fresh medium thus enormously diluting the original constituents of the medium or of the yeast. It can be concluded that after such a procedure any *B* present in the yeast must have been synthesized by the organism. Sufficient yeast was grown for feeding experiments on rats which made rapid gains when 2 per cent of the yeast grown on the synthetic medium was added to a ration free from *B* and on which the rats had begun to decline rapidly.[17]

Vitamins according to **Lumiére**[18] are not necessary to the development of plants, which reach full growth in media of known chemical composition. Extracts from substances rich in vitamins as dried raisins, aided development in media which were low in nutritive value, but their place could be taken perfectly by appropriate addition of mineral salts. Penicillium glaucum, Aspergillus niger, and Rhizopus nigricans and Amanita muscaria were used in these observations.

Willaman[19] has studied the metabolism of Sclerotinia cinerea, particularly with regard to the function of vitamins, and offers some interesting conclusions. Sclerotinia cinerea, the brown rot fungus of peaches and plums, cannot grow on a medium made up of sucrose, salts and asparagine. The addition to this medium of small amounts of plant decoctions, especially of the fruits of plums and peaches, induces growth. The experimental data reported in the paper show that the factor supplied by the plant decoctions is not one of mineral nitrogen or energy requirements but is of the nature of a vitamin. By means of absorption of fuller's earth, vitamin preparations were made from a large number of widely scattered sources, both plant and animal; all of these preparations were active in promoting growth in Sclerotinia and a few of them promoted reproduction also. Experiments designed to show whether two separate vitamin factors are in-

[17] Results obtained by **Fleming** (J. Biol. Chem. **49**, 119, 1921) are considered by him to disprove any specific action of water-soluble *B* in stimulation of yeast growth. The addition of organic nitrogen to the inorganic nitrogen of the culture medium employed apparently is in part at least responsible for the stimulation of yeast growth.

[18] Ann. inst. Pasteur **35**, 102, 1921.

[19] J. Am. Chem. Soc. 42, 549, 1920.

volved in the two phases, vegetation and reproduction, in the life history of Sclerotinia, yielded some evidence favoring this view: (1) The reproductive element is absorbed by fuller's earth more readily in an alcohol and the vegetative element more readily in an aqueous medium; (2) some sources yield preparations which predominate in the vegetative factor, others yield preparations that promote both activities; (3) preparations which promote only vegetation on a normal medium have not been induced to promote reproduction by changes in the proportions of the nutrients in the medium, and preparations that on normal media promote both activities have not been made to lose entirely the reproductive element, thus indicating that the influence of the nutrients in affecting the ratio of vegetation to reproduction is quantitative and not qualitative. On the other hand, the hypothesis of the existence of but a single vitamin for Sclerotinia is more plausible according to much of the experimental evidence. It is very probable that reproduction in the fungus is simply a different manifestation of the same activities as characterize vegetation. The single activity that is apparently most dependent on a vitamin supply is respiration. Respiration is common to all of the materials which have yielded the vitamin, and the degree of metabolic, and hence respiratory, activity in these materials is proportional to the activity of the vitamin prepared from them. Thus the evidence is accumulating in favor of the view that there is a close connection between respiration in a cell and its vitamin content, and also its vitamin requirement. Just which cells in the plant world can synthesize this vitamin is still an open question. The Sclerotinia vitamin is possibly identical with the water-soluble *B* of the higher animals, and since the latter cannot synthesize this vitamin it becomes important to know which plant organs can effect such a synthesis.[20]

A study of the effects of vitamins on the growth of *Oidium lactis,* made by **Linossier,**[21] afforded yields of growth, in mg., with and without the addition of vitamins to the medium respectively as follows: 2nd day 550; 4th day 157,218; 6th day 318,334; 9th day 365,345.

Goy[22] experimented with various fungi and bacilli and came to the conclusion that vitamins are not essential to the lower plant forms, but that the evolution of these is somewhat influenced by a nitrogen-free acid isolated in crystalline form from cultures of Mucor mucedo. The validity of this statement is questioned by **Funk**[23] on the ground that the substance actually isolated was entirely inactive.

[20] Chem. Abs. 1920, 1700.

[21] Compt. rend. soc. biol. 82, 381, 1919; Chem. Abs. 1920, 3436.

[22] Goy, C. r. 172, 242, 1921.

[23] Funk and Dubin, J. Biol. Chem. 48, 441, 1921.

Several investigators have reported on the necessity of vitamins for bacterial growth. **Davis**[24] found that the influenza bacillus requires for its nutrition a vitamin-like substance which is produced by a number of other bacteria and can be isolated from many animal and vegetable tissues.[25] Davis suggests that the rôle of the vitamins in the development of the bacilli is to render iron, calcium, phosphorus, iodine, and amino acids available for the metabolism of these organisms.[26]

Lloyd[27] states that in the absence of vitamins, meningococcus fails to develop, owing to its inability to utilize the amino acids of proteins. The necessary vitamins can be obtained from the body fluids.

Cole and Lloyd[28] found vitamins, or some substance of similar nature, necessary for the successful cultivation of gonococcus.

In nasal secretion there is present some body which greatly accelerates the growth of the meningococcus on an artificial culture medium. Alone it is incapable of acting as a food or stimulant to the growth of this organism. The body is soluble in water, less so in alcohol, and very insoluble in ether. It has great heat-resisting power, being able to resist prolonged boiling. Boiling in the presence of strong hydrochloric acid for twelve hours did not destroy the substance. In addition to the meningococcus it also stimulates the growth of *B* typhosus, the pneumococcus, *B* coli communis, and other pathogenic organisms.[29]

Pacini and Russell assert the presence of a growth-promoting substances in cultures of typhoid bacilli.[30] It has long been recognized that in certain infectious diseases, growth is induced apparently by the infection. In typhoid fever, for example, the patient may grow from one to even several inches in height. It is now shown that the fat and protein-free extract of typhoid bacilli contains a vitamin which when added to the food causes rapid growth of rats on a diet on which otherwise they gradually lose weight and die. The extract gives the blue color with Folin's uric acid regeant which is given by vitamin-containing extracts prepared from yeast and other sources. Pacini

[24] **Davis**, J. Infect. Dis. **21**, 392, 1917; **23**, 248, 1918; J. Am. Med. Assn. **77**, 683, 1921.

[25] See also **Fildes**, Brit. J. Exper. Path. **2**, 16, 1921; **Thjotta and Avery**, J. Exp. Med. **34**, 97, 1921; **Rivers**, Bull. Johns Hopkins Hosp. **32**, 202, 1921.

[26] **Davis**, *loc. cit.*, also J. Infect. Dis. **4**, 73, 1907.

[27] **Lloyd**, J. Path. Bact. 21, 113, 1916.

[28] **Cole and Lloyd**, *Ib.* **21**, 267, 1916.

[29] **Shearer**, Lancet 1917, 1, 59. The presence of an accessory food factor in the nasal secretion and its action on the growth of the meningococcus and other pathogenic bacteria. (Preliminary paper).

[30] **Pacini and Russell**, J. Biol. Chem. **34**, 43, 1918.

and Wright indicate the bearing of these results on the question of a more scientific method of feeding in these cases.

A study of vitamins utilizable in the culture of bacteria, especially with respect to the bacillus of influenza (B. de Pfeiffer), has been made by **Agulhon** and **Legroux.**[31] The favoring action of blood, serum, ascitic fluid, etc., upon bacterial cultures is accounted for by the presence of vitamins of growth in these liquids and not by the introduction of intact albumin into the cultures. To prepare extracts of blood rich in vitamins the defibrinated blood was precipitated with four volumes of absolute alcohol which was allowed to remain in contact 30 minutes and then removed by the centrifuge or filter. The precipitate was taken up with a volume of physiological saline solution equal to that of the alcohol used, allowed contact for one hour with agitation, then filtered or centrifuged. These operations were made aseptically or the solution was passed through the Chamberland F bougie. The solution thus obtained was nearly colorless and was rich in vitamins. In a second method the defibrinated blood was treated with four volumes physiological saline solution, heated 15 minutes on the water bath at 80° with frequent agitation, and filtered or centrifuged as in the first method. Solutions of vitamins obtained by either of these methods, when added to the usual culture media in amounts of 5 to 10 per cent, promote the growth of B. de Pfeiffer. Even a one per cent solution showed positive results. Experiments seemed to prove that the vitamins are fixed in the cells and a destruction of these elements by alcohol, heat or otherwise, sets them free in the aqueous liquid. They are probably insoluble in alcohol. A cold alcoholic extract of freshly washed cells was inactive but when made at 80° was slightly active. Cold or warm acetone extracts were inactive. Heating at 80° in the presence of alcohol or acetone followed by desiccation at a lower temperature did not destroy the vitamins, as they could be extracted with water from the powders thus obtained. Aqueous solutions of vitamins lose a part of their activity by heating 15 minutes at 90°. This activity is more persistent if the vitamins are in a gelose medium, when a decrease is noted at 100° and total disappearance at 120°.[32]

McLeod and **Wyon**[33] attempted to investigate the relations between vitamins and bacterial growth, with a view to the use of this as an index of the presence of vitamins. The medium used was a phosphate agar, to which various extracts and chemical substances

[31] Compt. rend. 167, 597, 1918.

[32] Chem. Abs. 1919, 855.

[33] **McLeod** and **Wyon**, J. Path. Bact. 24, 205, 1921.

were added. Especially striking results were obtained with kidney extract, minute amounts of which were effective in a manner suggestive of vitamin activity. A comparison of the chemical and physical properties of the staphylococcus-stimulant from yeast with those of vitamin *B* revealed serious discrepancies.[34] The growth-promoting power of fresh blood or serum does not bear any definite ration to the known vitamin content. Heating a serum partially destroys its growth-promoting power for the pneumococcus but not for the meningococcus.

[34] See also **Funk** and **Dubin,** J. Biol. Chem. **48,** 438, 1921.

APPENDIX

DISTRIBUTION OF VITAMIN A

ANIMAL PRODUCTS

FATS

Substance	Reference	Remarks
Beef fat	Osborne and Mendel, J. Biol. Chem. 20, 379, 1915.	18 % beef fat in diet induced far better growth than lard, but inferior to butter-fat.
Beef oil	Osborne and Mendel, J. Biol. Chem. 20, 379, 1915.	6% oil more effective than 18% fat, but inferior to corresponding amount of butter oil.
	Halliburton and Drummond, J. Physiol. 51, 235, 1917.	Confirm Osborne and Mendel as to presence of *A*.
Butter-fat	Osborne and Mendel, J. Biol. Chem. 15, 311, 1913.	18% of butter-fat in diet induces good growth in rats over long periods.
	Osborne and Mendel, J. Biol. Chem. 16, 423, 1913-14.	
	Osborne and Mendel, J. Biol. Chem. 17, 401, 1914.	
	Osborne and Mendel, J. Biol. Chem. 20, 379, 1915.	Secured good growth over long periods with 1% or 3% butter-fat in diet, but eventually animals declined unless amount was increased.
	Osborne and Mendel, J. Biol. Chem. 41, 549, 1920. (See also Mendel, N. Y. State J. Med. 20, 212, 1920; J. Am. Med. Ass. 75, 568).	0.1 g. (1.0-1.4% of diet) butter-fat daily enables rats to reach adult size before they show lack of *A*, but eventually they decline. It has repeatedly been observed, however, that 0.5 g. per day is sufficient to restore rats which have failed for lack of *A* to good nutritive condition, provided other dietary factors are satisfactory.
	McCollum and Davis, J. Biol. Chem. 15, 167, 1913.	10% butter-fat in diet caused good growth.
	McCollum and Davis, J. Biol. Chem. 20, 641, 1915.	Normal growth and reproduction with 5% butter-fat in diet.
	McCollum, Simmonds, and Parsons, J. Biol. Chem. 37, 162, 1919.	2% in diet sufficient for maintenance of good growth when all other dietary factors are of good quality. (According to Osborne and Mendel this probably represents from 150-200 mg. actually eaten.
	Drummond, Bioch. J. 13, 81, 1919.	Failed to secure normal growth of rats with 4% butter-fat in an otherwise suitable diet.

Butter-oil	Osborne and Mendel, J. Biol. Chem. 20, 379, 1915.	Higher concentration of *A* than butter-fat.
Egg, boiled, ether extract	McCollum and Davis, Proc. Soc. Exp. Biol. Med. 11, 101, 1914.	*A* present.
Egg-yolk, ether extract	McCollum and Davis, J. Biol. Chem. 15, 167, 1913.	1% in diet induced normal growth and reproduction in one rat, 5% little if any improvement.
	Osborne and Mendel, J. Biol. Chem. 17, 325, 401, 1914. See also Aron and Gralka, Biochem. Zeits. 115, 188, 1921.	As efficient as butter-fat.
Fish Oils		
Basking-shark liver	Sekine, J. Tok. Chem. Soc. 41, 426.	*A* present.
Cochin fish	Drummond, J. Physiol. 52, 103, 1919.	*A* present.
Cod liver	Osborne and Mendel, J. Biol. Chem. 17, 401, 1914.	6% in diet sufficient to induce normal growth in rats.
Cod liver (crude)	Drummond, l. c. See also Aron and Gralka, Biochem. Zeits. 115, 188, 1921.	Very rich in *A*.
Cod liver (raw, medicinal)	Drummond, l. c.	Very rich in *A*.
Cod oil (Norwegian oil, prepared from the bodies of cod after removal of livers)	Drummond, l. c.	*A* present.
Herring	Drummond, l. c.	*A* present.
Herring (Japan)	Drummond, l. c.	*A* present.
Menhaden	Drummond, l. c.	*A* present.
Salmon	Drummond, l. c.	*A* present.
Shark liver	Drummond, l. c.	*A* present.
Tunny liver	Sekine, l. c.	*A* present.
Whale	Drummond, l. c.	*A* present.
Lard	McCollum and Davis, J. Biol. Chem. 15, 167, 1913.	Diets containing 20% of lard gave practically normal growth for periods varying 70 to 120 days, then growth ceased.
	Osborne and Mendel, J. Biol. Chem. 15, 311, 1913. Osborne and Mendel, J. Biol. Chem. 16, 423, 1913-14. Osborne and Mendel, J. Biol. Chem. 20, 379, 1915.	Rats on a diet containing 28% of lard ceased to grow normally within 3 months unless provided with additional *A*. Different individuals vary greatly, however. One animal continued to thrive for 252 days before decline set in; in others growth stopped in 60 days.
	Halliburton and Drummond, J. Physiol. 51, 235, 1917.	No *A* present.

FATS (Continued)

Substance	Reference	Remarks
	Daniels and Loughlin, J. Biol. Chem. 42, 359, 1920.	28% of lard gave sufficient *A* for normal growth and reproduction over a period of about 5 months. On 21% the animals remained normal for about 2 months.
Liver oil, pig	Osborne and Mendel, J. Biol. Chem. 34, 17, 1915.	10% in diet induced prompt recovery in animals suffering from lack of *A*.
"Oleo oil"	Halliburton and Drummond, J. Physiol. 51, 235, 1917.	*A* present, but less than butter-fat.
Oleomargarine from animal fats	Osborne and Mendel, J. Biol. Chem. 20, 379, 1915.	Effective in restoring rats which had declined on diet lacking *A*.
	Halliburton and Drummond, l. c.	Equal to butter-fat in content of *A*.
MISCELLANEOUS		
Cheese, whole milk	Med. Res. Com. Sp. Rep. 38, p. 22.	Fairly rich in *A*.
Cheese, skim milk	Med. Res. Com. Sp. Rep. 38, p. 22.	No *A*.
Casein, or caseinogen (commercial)	Drummond and Coward, Bioch. J. 14, 661, 1920.	Relatively large amounts of *A*.
Egg white	Drummond and Coward, Bioch. J. 14, 661, 1920.	Relatively large amounts of *A*.
Fish		
Cod testicle, ether ext.	McCollum and Davis, J. Biol. Chem. 20, 641, 1915.	3% in diet equivalent in diet to butter-fat.
Roe	Med. Res. Com. Sp. Rep. 38, p. 22.	*A* present.
Shya-chi-no, flesh	Sekine, J. Tok. Ch. Soc. 41, 426.	*A* present.
Tunny, abdom. muscle	Sekine, J. Tok. Ch. Soc. 41, 426.	*A* present.
Heart, pig, dried	McCollum and Davis, J. Biol. Chem. 21, 179, 1915.	25% in diet causes some improvement in growth, but very inferior to kidney.
	Osborne and Mendel, J. Biol. Chem. 34, 17, 1918.	19% added to diet containing 23% lard caused fairly good growth, substitution of butter-fat for 9% of lard caused improvement.
Kidney, pig, dried	McCollum and Davis, J. Biol. Chem. 21, 179, 1915.	25% in diet otherwise free from *A* induces vigorous growth.
Kidney, pig, dried	Osborne and Mendel, J. Biol. Chem. 34, 17, 1918.	22% in diet containing 23% lard induced excellent growth.
Kidney, pig, ether extr.	McCollum and Davis, J. Biol. Chem. 20, 641, 1915.	Considerable amount of *A* present.

Meat extract (Com.)	Med. Res. Com. Sp. Rep. 38, p. 22.	*A* absent.
Pancreas	Emmett and Luros, J. Biol. Chem. 38, 441, 1919.	No *A* could be extracted with either acetone or benzene.
Thymus gland	Emmett and Luros, J. Biol. Chem. 38, 441, 1919.	No *A* could be extracted with either acetone or benzene.
Suprarenal gland	Emmett and Luros, J. Biol. Chem. 38, 441, 1919.	No *A* could be extracted with either acetone or benzene.

FATS

VEGETABLE PRODUCTS

Almond oil	Osborne and Mendel, J. Biol. Chem. 17, 401, 1914.	18% in diet fails to restore growth in rats which have been deprived of *A*.
Arachis (peanut) oil	Hale and Drummond, J. Physiol. 51, 235, 1917.	Little or no *A* present.
Coconut oil	Halliburton and Drummond, J. Physiol. 51, 235, 1917.	Little or no *A* present.
	Jansen, Geneesk. Tyd. v. Ned. Ind. 58, 173, 1920.	Very little *A* present. In same category as olive oil.
Corn oil	McCollum, Simmonds and Pitz, J. Biol. Chem. 25, 111, 1916.	Some, although little, *A* present.
Cottonseed oil	McCollum and Davis, J. Biol. Chem. 20, 641, 1915.	5% in diet has no demonstrated effect on growth.
	McCollum, Simmonds and Pitz, Am. J. Physiol. 41, 361, 1916.	Lacking in *A*.
	Halliburton and Drummond, J. Physiol. 51, 235, 1917.	Little or no *A* present.
	McCollum, Simmonds and Parsons, J. Biol. Chem. 33, 411, 1918.	8% in the diet provided insufficient *A* for growth of rats.
	Daniels and Loughlin, J. Biol. Chem. 42, 359, 1920.	28% in the diet permitted normal growth and reproduction in rats for 4 months. 21% was much less satisfactory, but vigorous animals grew normally for about 6 weeks and then declined.
Hydrogenated oils	Halliburton and Drummond, J. Physiol. 51, 235, 1917.	Lacking in *A*.
Lard substitutes	Halliburton and Drummond, l. c.	No *A* present.

FATS (Continued)

Substance	Reference	Remarks
Linseed oil	McCollum, Simmonds and Pitz, Am. J. Physiol. 41, 361, 1916.	Lacking in *A*.
Margarines from vegetable oils	Halliburton and Drummond, J. Physiol. 51, 235, 1917.	*A* insufficient to support growth for more than a short period.
Olive oil	McCollum and Davis, J. Biol. Chem. 15, 167, 1913; 19, 248, 1914; 20, 641, 1915.	On a diet with 5% olive oil rats continued to grow at slightly subnormal rate for 6 months, but no young were produced. Note that vigorous rats have accomplished very similar records on fat-free diets.
Palm oil	Drummond and Coward, Bioch. J. 14, 668, 1920.	Good source of *A*.
Peanut oil	(See arachis oil).	
Sunflower seed oil	McCollum, Simmonds and Pitz, Am. J. Physiol. 41, 361, 1916.	Lacking in *A*.

LEAFY VEGETABLES AND GRASSES

Substance	Reference	Remarks
Alfalfa	McCollum, J. Am. Med. Ass. 68, 1379, 1917.	30% in diet supplies enough *A* to keep animal in normal condition if diet is otherwise adequate.
	McCollum, Simmonds and Pitz, J. Biol. Chem. 30, 13, 1917.	Satisfactory source of *A*.
	Osborne and Mendel, *Ib.* 37, 187, 1919; 41, 549, 1920.	Very small amounts furnish sufficient *A* for long-continued and vigorous growth. Is at least as efficient, relatively, as butter-fat, possibly more so.
	Steenbock and Gross, *Ib.* 41, 149, 1920.	Normal growth and reproduction possible on a diet in which 5% of alfalfa (cut in full bloom) represents the sole source of *A*.
Cabbage, dried at 60°	Osborne and Mendel, J. Biol. Chem. 37, 187, 1919; 41, 549, 1920.	15% in diet furnishes sufficient *A* for normal growth. Less satisfactory than spinach or carrots.
Dried at room temperature	Steenbock and Gross, J. Biol. Chem. 41, 149, 1920.	15% in diet induced maintenance and subnormal growth for 26 weeks, but addition of butter-fat caused improvement.
Fresh, absolute Alcohol extr.	Zilva, Bioch. J. 14, 494, 1920.	Extract equivalent to 25 g. of fresh cabbage per day induced some growth in rats, but experiment was inconclusive owing to unpalatability of extract.

Celery leaf	McCollum, J. Am. Med. Ass. 68, 1379, 1917.	30% in diet supplies enough *A* to keep an animal in normal condition, if diet is otherwise adequate.
Chard	Steenbock and Gross, J. Biol. Chem. 41, 149, 1920.	5% in diet furnishes sufficient *A* for long-continued but slightly subnormal growth. Reproduction was poor.
Clover	McCollum, J. Am. Med. Ass. l. c.	*A* present.
	Osborne and Mendel, J. Biol. Chem. 37, 187, 1919; 41, 549, 1920.	Furnishes relatively as much *A* as butter-fat, possibly more. Very small amounts furnish sufficient *A* for long-continued and vigorous growth.
	Steenbock and Gross, *Ib.* 41, 149, 1920.	5% of clover cut at end of blossoming period furnished sufficient *A* to permit of approximately normal growth and slightly subnormal reproduction.
Grass	Osborne and Mendel, J. Biol. Chem. 41, 549, 1920.	42 m. g. per day of ethereal extract furnished sufficient *A* to promote growth of rats which had declined for lack of it (150-200 m. g. of butter-fat required to effect same result).
Lettuce, air dried	Steenbock and Gross, J. Biol. Chem. 41, 149, 1920.	5% in ration as sole source of *A* permits of long-continued but somewhat subnormal growth. Reproduction poor.
Spinach, dried at 60°	McCollum, J. Am. Med. Ass. 68, 1379, 1917.	30% in diet supplies enough *A* to keep animal in normal condition, if diet is otherwise adequate.
	Osborne and Mendel, J. Biol. Chem. 37, 187, 1919; 41, 549, 1920.	Furnishes relatively as much *A* as butter-fat, possibly more. Richer than most of the products used in ordinary rations.
	Steenbock and Gross, J. Biol. Chem. 41, 149, 1920.	5% in ration furnishes sufficient *A* for long-continued though somewhat subnormal growth. Reproduction poor.
Timothy hay	Osborne and Mendel, J. Biol. Chem. 37, 187, 1919; 41, 549, 1920.	Very small amounts furnish sufficient *A* for long-continued and vigorous growth. Relatively as much *A* as butter-fat, possibly more.

CEREAL GRAINS AND SEEDS

Barley	Steenbock, Kent, and Gross, J. Biol. Chem. 35, 61, 1918.	Somewhat deficient in *A*, 15% of barley in a diet otherwise free from *A* has no effect on growth. 60% improves.
Beans, Kidney	Med. Res. Com. Rep. 38, p. 22.	*A* present.
Navy	McCollum and Simmonds, J. Biol. Chem. 32, 29, 1917.	Little if any *A* present.

CEREAL GRAINS AND SEEDS (Continued)

Substance	Reference	Remarks
Beans, Soy	Osborne and Mendel, *Ibid.* 32, 369, 1917.	*A* present, but different soy bean meals were found to differ considerably in this respect, probably owing to method of preparatory treatment.
	Daniels and Nichols, *Ib.* 32, 91, 1917.	*A* present, to some extent at least.
White (Phaseolus vulgaris)	McCollum, Simmonds, and Pitz, *Ib.* 29, 521, 1918.	50% in ration induced no growth in rats in 8 weeks.
Cereal grain mixtures	McCollum and Simmonds, J. Biol. Chem. 33, 303, 1918.	Mixtures of wheat, maize, and oats proved too poor in *A* to maintain growth.
Corn (See maize)		
Cotton-seed meal	Richardson and Green, J. Biol. Chem. 31, 379, 1917.	4.35% ether extract of cottonseed flour, equivalent to 50% of cottonseed flour in diet, does not contain sufficient *A* for normal growth, but 12% of the ether extract appears quite as sufficient as an equivalent amount of butter-fat.
Flax seed	McCollum, Simmonds, and Pitz, J. Biol. Chem. 30, 13, 1917.	Distinctly richer than wheat, oats, or maize.
Hemp seed	McCollum, Simmonds, and Pitz, *Ib.* 30, 13, 1917; 33, 303, 1918.	Richer than the cereal grains, but less so than flax or millet.
Lentils	Chick and Delf, Bioch. J. 13, 199, 1919.	Apparently deficient in *A*.
Linseed	Med. Res. Com. Rep. 38, p. 22.	Fairly rich in *A*.
Linseed cake after expulsion of oil	Med. Res. Com. Rep. 38, p. 22.	*A* present.
Maize meal	McCollum and Davis, J. Biol. Chem. 21, 179, 1915.	5% not sufficient to prevent death from lack of *A*; 50% more efficient than 5% of butter-fat.
Kernel	McCollum, Simmonds, and Pitz, J. Biol. Chem. 28, 153, 1916; Am. J. Physiol. 41, 374, 1916.	*A* present in amounts too small for maintenance and growth when this grain forms the chief content of the diet for rats.
White	Steenbock and Boutwell, J. Biol. Chem. 41, 81, 1920.	No demonstrable amount of *A*.
Yellow	Steenbock and Boutwell, J. Biol. Chem. 41, 81, 1920.	Provides for normal growth in rat when fed to extent of 88% of diet. Reproduction possible, but usually a failure.
	Steenbock, Boutwell, and Kent, *Ib.* 41, xii, 1920.	On yellow maize supplemented with protein and salts young rats have grown to maturity, maintained themselves for months, and repeatedly reproduced.

Millet seed	McCollum, Simmonds, and Pitz, J. Biol. Chem. 30, 13, 1917.	Distinctly richer than the cereal grains.
Oats	McCollum and Davis, J. Biol. Chem. 21, 179, 1915.	Inferior to maize in content of *A*.
	McCollum, Simmonds, and Pitz, *Ib*. 29, 341, 1917.	Very small amount of *A* present. Growth slow even where supplemented with 5% butter-fat.
Peas, dried	McCollum, Simmonds, and Parsons, J. Biol. Chem. 33, 411, 1918.	Over 50% in diet furnishes insufficient *A* for growth of rats.
	Chick and Delf, Bioch. J. 13, 199, 1919.	Deficient in *A*.
Rice, polished	Guerrero and Concepcion, Phil. J. Sci. 17, 99, 1920.	Deficient in *A*, as shown by appearance of xerophthalmia in fowls.
Polishings	Med. Res. Com. Rep. 38, p. 22.	*A* present.
Rye	McCollum and Davis, J. Biol. Chem. 21, 179, 1917.	Probably a little *A* present but very little. Addition of 50% of rye to a diet deficient in *A* caused gain in weight, but otherwise no improvement in condition.
Tomato-seed press cake	Finks and Johns, Am. J. Physiol. 56, 404, 1921.	Can serve as sole source of *A*.
Wheat		
Whole kernel	Hart and McCollum, J. Biol. Chem. 19, 373, 1915.	Somewhat deficient in *A*.
	McCollum and Davis, *Ib*. 21, 179, 1915.	5% in diet insufficient for maintenance; 50% insufficient for growth.
Embryo	McCollum and Davis, *Ib*. 21, 179, 1915.	50% in diet induces prompt recovery in rats which have declined for lack of *A*.
	McCollum, Simmonds, and Pitz, *Ib*. 25, 105, 1916.	33% in diet insufficient for growth of rats, but 5% of *ether extracted* embryo as sole source of *A* induced nearly normal growth for 3 to 4 months. (Explained as due to removal of toxic substance by ether, resulting in lowering of vitamin requirement at least temporarily).
Patent flour	McCollum, Simmonds, and Parsons, *Ib*. 33, 411, 1918.	25% in diet insufficient to permit of growth in rats.
	Voegtlin and Myers, Pub. Health Rep. 33, No. 22, p. 843.	"White flour" deficient in *A*, but flour in which part of the germ and superficial layers of the grain have been retained support growth especially well.

TUBERS, ROOTS, ETC.

Substance	Reference	Remarks
Beet, red (dried)	Steenbock and Gross, J. Biol. Chem. 40, 501, 1919; Sci. 50, 352, 1919.	15% in ration did not provide demonstrable amount of *A*.
Sugar (dried)	Steenbock and Gross, J. Biol. Chem. 40, 501, 1919; Sci. 50, 352, 1919.	25% in ration did not provide demonstrable amount of *A*.
Carrot (dried)	Steenbock and Gross, J. Biol. Chem. 40, 501, 1919; Sci. 50, 352, 1919.	Remarkably rich in *A*. 15% in ration as sole source of *A* permits successful rearing of young by mother.
	Osborne and Mendel, J. Biol. Chem. 41, 549, 1920.	Less effective than spinach.
	Zilva, Bioch. J. 14, 494, 1920.	Abs. alc. extract equivalent to 10 to 12 g. fresh carrots per day gives sufficient *A* for normal growth of rats. Ethereal ext. from alcoholic fraction equivalent to 25 g. of fresh carrots promoted recovery and renewed growth in rats declining from lack of *A*.
Dasheen (dried)	Steenbock and Gross, J. Biol. Chem. 40, 501, 1919.	No demonstrable amount of *A* present when fed to extent of 15% of ration.
Mangel	Steenbock and Gross, J. Biol. Chem. 40, 501, 1919.	25% in ration does not provide enough *A* for normal growth of rat.
Parsnip	Steenbock and Gross, J. Biol. Chem. 40, 501, 1919.	Poor in *A*.
Potatoes (cooked)	Steenbock and Gross, J. Biol. Chem. 40, 501, 1919.	Great variation in different lots of potatoes. May contain enough *A* for normal growth if fed at high level, but generally can be considered poor in this essential.
	McCollum, Simmonds, and Parsons, *Ib*. 36, 197, 1918.	About equal to cereal grains as source of *A*.
Potatoes, dried at 60°	Osborne and Mendel, *Ib*. 41, 549, 1920.	Poor in *A*, but not devoid of it.
Potatoes, sweet, dried at 50-60°	Steenbock, Sci. 50, 352, 1919.	Rich in *A*.
	Steenbock and Gross, J. Biol. Chem. 40, 501, 1919.	15% in ration permits long-continued growth and rearing of young.
Rutabaga (Swedish turnip)	Steenbock and Gross, J. Biol. Chem. 40, 501, 1919.	Little *A*, if any.
Squash, Hubbard	Steenbock and Boutwell, J. Biol. Chem. 41, 170, 1920.	Considerable amount of *A* present.
Turnip (See Rutabaga)		

FRUITS AND NUTS		
Apples, green	Med. Res. Com. Rep. 38, p. 22.	*A* absent.
Bananas	Suguira and Benedict, J. Biol. Chem. 36, 171, 1918.	*A* present.
Grape fruit juice	Osborne and Mendel, J. Biol. Chem. 42, 465, 1920.	Equivalent of 10 cc. per day insufficient to prevent appearance of xerophthalmia.
Lemon juice	Osborne and Mendel, J. Biol. Chem. 42, 465, 1920.	Equivalent of 10 cc. per day insufficient to prevent appearance of xerophthalmia.
Orange juice	Drummond and Coward, Bioch. J. 14, 661, 1920.	Completely devoid of *A*.
Tomato, dried at 60°	Mendel, J. Am. Med. Ass. 75, 568; N. Y. State J. Med. 20, 212, 1920. Osborne and Mendel, J. Biol. Chem. 41, 549, 1920.	Better than butter-fat as source of *A*, 0.1 g. (representing 1.0-1.4% of the ration) sufficed as source of *A* for rats for 14 months. Note, however, that ether extract of tomato was not effective.
Coconut press-cake	Johns, Fink, and Paul, J. Biol. Chem. 37, 497, 1919.	Some *A* present, but not optimum amount for growth.
Nuts	Coward and Drummond, Bioch. J. 14, 665, 1920.	Common varieties of edible nuts of little value as source of *A*.
Peanuts	Daniels and Loughlin, J. Biol. Chem. 33, 295, 1918.	Lacking in *A*.
MISCELLANEOUS		
Custard powders and egg substitutes	Med. Res. Com. Rep. 38, p. 22.	*A* absent.
Honey (in comb)	Hawk, Smith and Bergheim, Am. J. Physiol. 55, 339, 1921.	Moderate amounts of *A* present.
Strained	*Ib.*	Not more than minimal amount of *A*.
Malt extract	Med. Res. Com. Rep. 38, p. 22.	*A* absent.
Yeast	Drummond, Bioch. J. 11, 255.	Free from *A*.
Dried brewery	Osborne and Mendel, J. Biol. Chem. 31, 158, 1917; 37, 199, 1918.	Free from *A*.

DISTRIBUTION OF VITAMIN B

ANIMAL PRODUCTS

MEAT AND FISH

Substance	Reference	Remarks
Fat, pork	Sullivan and Voegtlin, J. Biol. Chem. 24, xvi, 1915-16.	Antineuritic absent.
Glandular tissue		
Brain, ox	Cooper, J. Hyg. 12, 433, 1912; 14, 12, 1914.	Effective as antineuritic, though less so than liver. 6 g. per day gave protection.
Pig Dried at 90°	Osborne and Mendel, J. Biol. Chem. 34, 17, 1918.	32.5% in diet furnished sufficient *B* for normal growth, 10% inadequate.
Sheep	Cooper l. c.	Less efficient than ox-brain as antineuritic. 6 g. per day gave protection.
Glandular organs of herring	Drummond, J. Physiol. 52, 103, 1919.	Small amounts of *B* present.
Heart, ox	Drummond, J. Physiol. 52, 103, 1919.	Contains some antineuritic, but less than liver or brain.
Pig, dried at 90°	Osborne and Mendel, J. Biol. Chem. 34, 17, 1918.	19% in diet gave enough *B* for normal growth.
Kidney, pig, dried at 90°	Osborne and Mendel, J. Biol. Chem. 34, 17, 1918.	22% in diet gave enough *B* for normal growth.
Dog	Swoboda, *Ib.* 44, 539, 1920.	Very high in growth-promoting *B* (yeast test).
Liver		
Ox	Cooper, l. c.	High in antineuritic.
Pig, dried at 90°	Osborne and Mendel, l. c.	10% in diet furnished sufficient, 5% insufficient *B* for normal growth.
Ox	Sullivan and Voegtlin, J. Biol. Chem. 24, xvi, 1915; Voegtlin, Lake, and Myers, U. S. Pub. Health Rep. 33, 647, 1918.	Antineuritic present. Can be used as source of concentrated preparations.
Ox	Swoboda, l. c.	Relatively high in growth-promoting *B* (yeast test).
Dog	Swoboda, l. c.	Much lower than ox liver (yeast test).
Lymph gland	Swoboda, l. c.	Traces only of liver (yeast test).
Myocardium	Cooper, l. c.	Fairly satisfactory as antineuritic, although less so than liver.
Ovarian tissues	Drummond, Bioch. J. 12, 25, 1918.	Deficient in *B*.
	Swoboda, J. Biol. Chem. 44, 539, 1920.	Fairly high in growth-promoting *B* (yeast test).

Pancreas	Eddy, J. Biol. Chem. 27, 113, 1916-17.	Water extract contains considerable growth-promoting *B*.
	Williams, *Ib*. 38, 473, 1919.	Considerable amount of growth-promoting *B* (yeast test).
Dog	Swoboda, l. c.	Exceedingly low in growth-promoting *B* (yeast test).
Pineal gland, cow	Swoboda, l. c.	Fairly high in *B*, richer than pituitary (yeast test).
Pituitary, dog's whole	Swoboda, l. c.	Fairly high in *B* (yeast test).
Anterior lobe	Swoboda, l. c.	Fairly high in *B* (yeast test).
Pars intermedia	Swoboda, l. c.	Fairly high in *B* (yeast test).
Posterior lobe	Swoboda, l. c.	Fairly low in *B* (yeast test).
Colloid	Swoboda, l. c.	Fairly low in *B* (yeast test).
Whole gland, dried	Drummond, Bioch. J. 12, 25, 1918.	Deficient in *B*.
Anterior lobe, dried	Drummond, Bioch. J. 12, 25, 1918.	Deficient in *B*.
Roe, turbot	Chick and Hume, J. Roy. Army Med. Corps, 29, 121, 1917.	Alcoholic extract equivalent to 70 g. material sufficient, 35 g. insufficient to cure polyneuritic pigeon.
Sciatic nerve, dog	Swoboda, l. c.	Considerable amount of *B* present (yeast test).
Supravenal, dog	Swoboda, l. c.	Considerable amount of *B* present (yeast test).
Testes	Swoboda, l. c.	Large quantities of *B* present (yeast test).
	Drummond, l. c.	Deficient in *B*.
Thymus	Drummond, l. c.	Deficient in *B*.
	Swoboda, l. c.	Relatively little *B* (yeast test).
Glandular tissues		
Thyroid	Drummond, Bioch. J. 12, 25, 1918.	Deficient in *B*.
	Swoboda, J. Biol. Chem. 44, 539, 1920.	Considerable quantities of *B* present (yeast test).
	Seaman, Am. J. Physiol. 53, 101, 1920.	Antineuritic present. Acid-alcohol extract has marked accelerating action on metamorphosis of tadpoles.
Muscle tissue		
Commercial extracts	Chick and Hume, quoted Med. Res. Com. Rep. 38, p. 29.	No antineuritic present.
Powders, etc.	Osborne and Mendel, J. Biol. Chem. 32, 309, 1917; Am. J. Physiol. 45, 539, 1917.	Little, if any, growth-promoting *B* present.
Aqueous extract	Osborne and Mendel, J. Biol. Chem. 32, 309, 1917; Am. J. Physiol. 45, 539, 1917.	May furnish enough *B* for considerable growth if given in quantity.
Beef, raw	Cooper, J. Hyg. 14, 12, 1914.	Rather poor in antineuritic. Alcoholic extract equivalent to 140 g. meat cured a polyneuritic pigeon.
Dried	Osborne and Mendel, J. Biol. Chem. 32, 309, 1917.	Little, if any, *B* present.

MEAT AND FISH (Continued)

Substance	Reference	Remarks
Fish Muscle	Cooper, J. Hyg. 12, 436; 14, 12, 1914.	Little, if any, antineuritic present. 10 g. per day afforded no protection against polyneuritis.
Cod	Drummond, J. Physiol. 52, 103, 1919.	No appreciable amount of *B* present.
Herring	Drummond, J. Physiol. 52, 103, 1919.	No appreciable amount of *B* present.
Salmon	Drummond, J. Physiol. 52, 103, 1919.	No appreciable amount of *B* present.

EGGS

Substance	Reference	Remarks
Eggs, whole commercial dried	Chick and Hume, quoted in Med. Res. Com. Rep. 38, p. 29.	Equivalent of 20 g. natural egg (about 2 yolks) effective cure for polyneuritis.
Egg yolk	Cooper, J. Hyg. 14, 12, 1914.	3 g. per day afforded protection against polyneuritis.
Boiled	McCollum and Davis, J. Biol. Chem. 23, 181, 1915.	Ag. extract equivalent to 6.4% of diet induced vigorous growth.
Yolk	Steenbock, J. Biol. Chem. 29, xxvii, 1917.	Water-acetone extract in small doses by intraperitoneal injection cured polyneuritis.

MILK AND MILK PRODUCTS

Substance	Reference	Remarks
Milk	Cooper, J. Hyg. 12, 433, 1912.	Far inferior to various animal tissues as antineuritic.
	Hopkins, J. Physiol. 44, 425, 1912.	35 g per day failed to give protection against polyneuritis.
	Hopkins and Neville, Bioch. J. 7, 97, 1913.	2 c. c. per day or equivalent in solids to 1 to 4% of food induced good growth.
	Drummond, Bioch. J. 10, 89, 1916.	Growth-promoting *B* present.
	Gibson and Concepcion, Phil. J. Sci. 11, B, 119, 1916.	Antineuritic present in small amounts only.
	Chick and Hume, J. Roy. Army Med. Corps, 29, 121.	Poor in antineuritic.
	McCollum and Davis, *Ib.* 23, 181, 1915.	As little as 2% of milk powder in the diet furnishes enough *B* for nearly normal growth.
	Osborne and Mendel, J. Biol. Chem. 34, 537, 1918.	Less than 24% of dried whole milk unsatisfactory for production of good growth.
	Osborne and Mendel, *Ib.* 41, 515, 1920.	15 c. c. of fresh unpasteurized summer milk inferior to 0.2 g dried brewers' yeast as source of *B* for growth.
	Williams, *Ib.* 46, 113, 1921.	Very poor in *B* (yeast test).
Butter-fat	McCollum and Kennedy, *Ib.* 24, 491, 1915-16.	Devoid of antineuritic.

Casein, or caseinogen (unpurified)	Osborne, Wakeman, and Ferry, J. Biol. Chem. 39, 35, 1919.	Almost if not completely devoid of growth-promoting *B*.
Casein (merck) hydrolyzed	Williams, *Ib*. 38, 473, 1919.	*B* absent (yeast test).
Lactalbumin	Osborne, Wakeman, and Ferry, J. Biol. Chem. 39, 35, 1919.	Practically no *B* retained in a preparation precipitated in acid solution.
Cheese	Chick and Hume, J. Roy. Army Med. Corps, 29, 121, 1917.	Poor in antineuritic.
Cheddar	Cooper, J. Hyg. 14, 12, 1914.	Devoid of antineuritic.
Lactose	McCollum and Davis, J. Biol. Chem. 23, 181, 231, 1915.	Carries growth-promoting *B* which can only be removed by careful fractional crystallization.
	Drummond, Bioch. J. 10, 89, 1916.	Carries growth-promoting *B* which can only be removed by careful fractional crystallization.
Lactose (Kahlbaum)	Williams, l. c.	Small amount of *B* (yeast test).
MISCELLANEOUS		
Bile, ox	Muckenfuss, J. Am. Chem. Soc. 40, 1606, 1918.	Antineuritic present, probably in small quantity.
Blood, plasma from jugular vein	Eddy and Stevenson, J. Biol. Chem. 43, 295, 1920.	*B* present (yeast test).
Plasma from mammary vein	Eddy and Stevenson, J. Biol. Chem. 43, 295, 1920.	Richer than jugular vein (yeast test).
Feces	Osborne and Mendel, Carn. Pub. 156, pt. 2, p. 61.	Rats declining on a vitamin-free diet improved when fed small quantities of feces from rats on normal diet.
	Cooper, J. Hyg. 14, 12, 1914.	Antineuritic present.
Saliva	Muckenfuss, l. c.	Useless as a preventive, but seemed to have some curative power for polyneuritis.
	Funk and Dubin, J. Biol. Chem. 44, 487, 1920.	No *B* present (yeast test).
Urine	Muckenfuss, l. c.	Antineuritic apparently present in traces in fresh human urine.
	Gaglio, Polyclinico, 26, 1381, 1919.	Pronounced polyneuritis cured by small amounts of human urine.
	Curatolo, *Ib*. 27, 439, 1920.	Confirms Goglio, using rabbit urine. Potency apparently restricted to nitrogenous waste in the urine.
	Funk and Dubin, l. c.	*B* present (yeast test).

LEAFY VEGETABLES AND GRASSES

Substance	Reference	Remarks
Alfalfa	McCollum, Simmonds, and Pitz, Am. J. Physiol. 41, 333.	*B* present.
	Osborne and Mendel, J. Biol. Chem. 41, 451, 1920.	Alfalfa surpasses all other products known, except clover (and possibly tomatoes) in growth-promoting power. 1 g. per day (10 to 15% of food intake) produced extraordinarily good growth.
	Steenbock and Gross, *Ib.* 41, 149, 1920.	10% in diet insufficient. 15% sufficient to induce normal growth.
	Williams, *Ib.* 46, 113, 1921.	Less than half the value of brewers' yeast (yeast test).
Cabbage, juice	McCollum and Kennedy, J. Biol. Chem. 24, 491, 1915-16.	Antineuritic present, though not in very large amounts.
	Chick and Hume, Tr. Soc. Trop. Med. Hyg. 10, 141, 1916-17.	Antineuritic present in rather low concentration as compared with whole wheat.
	Osborne and Mendel, J. Biol. Chem. 41, 451, 1920.	Comparable to spinach in growth-promoting power.
	Steenbock and Gross, *Ib.* 41, 149, 1920.	15% in diet furnishes sufficient *B* to promote normal growth.
	Williams, *Ib.* 46, 113, 1921.	Slightly less effective than spinach (yeast test).
Etiolated leaves	Delf, S. Af. J. Sci. 17, 121, 1920.	Growth-promoting *B* lacking.
Clover	Osborne and Mendel, l. c.	Equivalent to alfalfa.
	Steenbock and Gross, l. c.	10% in diet insufficient, 15% sufficient for normal growth, 20% still better.
"Greens"	Chick and Hume, Med. Res. Com. Rep. 38, p. 30.	Alc. extract corresponding to 120 g. of material completely cured a polyneuritic pigeon.
Spinach, dried at 50 to 60°	Osborne and Mendel, J. Biol. Chem. 37, 187, 1919; 41, 451, 1920.	About one-fourth as efficient as dried yeast, one-half as efficient as alfalfa, and twice as efficient as whole wheat, dried eggs, or milk solids in promoting growth. Less than 1 g. daily (10 to 15% of the food intake) insufficient for growth.
	Williams, *Ib.* 46, 113, 1921.	Less than one-third the value of brewers' yeast (yeast test).
Timothy hay	Osborne and Mendel, *Ib.* 41, 451, 1920.	Apparently potent in promoting growth in earlier periods of experiment, but disappointing in the long run.

CEREAL GRAINS AND SEEDS

Barley, unhusked	Cooper, J. Hyg. 12, 436; 14, 12.	Contains antineuritic.
	Chick and Hume, Med. Res. Com. Rep. 38, p. 28.	3.7 g. per day added to polished rice diet protected pigeons against polyneuritis.
Husked	Chick and Hume, Med. Res. Com. Rep. 38, p. 28.	5.0 g. per day added to polished rice diet protected pigeons against polyneuritis.
	Steenbock, Kent, and Gross, J. Biol. Chem. 35, 61, 1918.	When fed to extent of 60% of ration, normal growth and reproduction; 40%, normal growth, no young reared; 20%, growth not quite normal.
	Osborne and Mendel, *Ib.* 41, 275, 1920.	45% in diet furnished sufficient *B* for normal growth.
Pearled	Osborne and Mendel, *Ib.* 41, 275, 1920.	72% in diet furnished sufficient *B* for normal growth.
Beans, string, commercial, canned	Davis, J. Home Ec. 12, 209, 1920.	10 g. per day afforded no protection, but if supplemented by 2 g. raw corn gave complete protection against polyneuritis.
Soy	Daniels and Nichols, J. Biol. Chem. 32, 91, 1917.	Liberal amount of growth-promoting *B* present.
	Osborne and Mendel, Proc. Soc. Exp. Biol. Med. 14, 174, 1917.	Equivalent to milk in growth-promoting power.
	Johns and Finks, Am. J. Physiol. 55, 455, 1921.	15% soy bean flour and 85% wheat flour gave ample *B* for growth.
Oil	Fahrion, Chem. Umschau. 27, 97, 109, 1920.	Pressed oil may contain vitamin, oil extracted with solvent little or none.
White	McCollum, Simmonds, and Pitz, J. Biol. Chem. 29, 521, 1917.	25% in the diet furnishes sufficient *B* for normal growth and reproduction.
	Voegtlin, Lake, and Myers, U. S. Pub. Health Repts. 33, 647, 1918.	Concentrated preparation of antineuritic can be obtained.
Corn (see maize)		
Cottonseed flour	Osborne and Mendel, Proc. Soc. Exp. Biol. Med. 13, 147, 1916.	Equivalent to milk in growth-promoting power.
	Richardson and Green, J. Biol. Chem. 31, 379, 1917.	Aqueous extract equivalent to 50% of flour in the diet furnished sufficient *B* for normal growth for 90 days.
Maize kernel	Funk, Bioch. Bull. 5, 1, 1913.	Antineuritic vitamins probably distributed in external layers.
	Voegtlin, Lake, and Myers, U. S. Pub. Health Reps. 33, 647.	15 to 20 g. per day supplied sufficient *B* for prevention of polyneuritis.
	Green, S. Af. J. Sci. 14, 519, 1918.	60% more antineuritic than required in metabolism.

CEREAL GRAINS AND SEEDS (Continued)

SUBSTANCE	REFERENCE	REMARKS
Endosperm	Funk and Dubin, J. Biol. Chem. 44, 487, 1920.	*B* present (yeast test).
Degerminated	Voegtlin and Myers, J. Biol. Chem. 41, x, 1920.	Practically devoid of antineuritic.
Germ	Chick and Hume, Proc. Roy. Soc. 90B, 44, 1917.	Marked antineuritic potency present in both scutellum and plantlet. 1 to 3 g. effected cure of polyneuritic pigeon.
Germ, commercial	McCollum, Simmonds, and Pitz, J. Biol. Chem. 28, 153, 1916-17.	Growth-promoting *B* present in abundance.
Germ, alc. ext.	Voegtlin and Myers, J. Biol. Chem. 41, x, 1920.	Effective antineuritic.
Bran	Voegtlin, Lake, and Myers, l. c.	10 g. per day prevented onset of polyneuritis.
	Green, l. c.	80% in diet provided more antineuritic than required in metabolism.
Grits, highly milled	Voegtlin, Lake, and Myers, l. c.	15 to 20 g. daily failed to prevent polyneuritis.
Meal, old process water ground	Voegtlin, Lake, and Myers, l. c.	10 g. daily prevented polyneuritis, but weight was not maintained.
Fine meal	Green, l. c.	20% more antineuritic than required in metabolism.
Seconds	Green, l. c.	70% more antineuritic than required in metabolism.
Hominy	Voegtlin, Lake, and Myers, l. c.	15 to 20 g. daily prevented polyneuritis.
Hominy chop.	Green, l. c.	Very rich in antineuritic.
Hominy samp.	Green, l. c.	Very poor in antineuritic.
Starch	Osborne, Wakeman, and Ferry, J. Biol. Chem. 39, 35, 1919.	No appreciable quantity of growth-promoting *B*.
Oats	McCollum and Kennedy, J. Biol. Chem. 24, 491, 1915-16.	Pigeon cured of polyneuritis and kept in good condition by ag. ext. of 24 g. rolled oats per day.
	McCollum, Simmonds, and Pitz, *Ib*. 29, 341, 1917.	Oat kernel contains a liberal supply of *B*.
	Osborne and Mendel, *Ib*. 41, 283, 1920.	35.5% of oat kernels in the diet furnish sufficient *B* for normal growth.
Peas, dried	Chick and Hume, Med. Res. Com. Rep. 38, p. 30.	10 g. effected complete cure of polyneuritic pigeon.
	Vedder and Clark, Phil. J. Sci. 7B, 423.	10 g. daily protects fowls on polished rice diet from polyneuritis for at least 60 days.

	McCollum, Simmonds, and Parsons, J. Biol. Chem. 37, 287, 1919.	5% in diet failed to protect rats against polyneuritis. (Compare with wheat germ, of which 2% is sufficient).
Cow	Sullivan and Voegtlin, J. Biol. Chem. 24, xvi, 1915-16.	Antineuritic present.
Rice, unhusked	Gibson and Concepcion, Phil. J. Sci. 9B, 119, 1914.	Exclusive diet afforded incomplete protection against polyneuritis, as shown by degenerative changes of sciatic nerve.
	McCollum and Davis, J. Biol. Chem. 23, 206, 1915.	Comparable to whole wheat. 88% in the diet furnishes sufficient *B* for normal growth.
Polished	Eijkman, Arch. path. Anat. 148, 523, 1897; 149, 187; Arch. Hyg. 58, 150, 1906.	Devoid of antineuritic.
	Suzuki, Shimamura, and Odake, Bioch. Z. 43, 89, 1912.	
	Funk, Ergeb. Physiol. 13, 125, 1913.	
Rice polishings	Eijkman, Arch. path. Anat. 148, 523, 149, 187, 1987.	Antineuritic present.
	Fraser and Stanton, Phil. J. Sci. 5B, 55, 1910.	
	Chamberlain and Vedder, Phil. J. Sci. 6B, 251, 395, 1911.	
	Funk, J. Physiol. 43, 395, 1911-12.	
	Suzuki, Shimamura, and Odake, Bioch. Z. 43, 89, 1912.	
	Edie, Evans, Moore, Simpson, and Webster, Bioch. J. 6, 234, 1912.	
	Cooper, *Ib*. 7, 268, 1912-13.	
	Strong and Crowell, Tr. 15th Int. Cong. Hyg. 5, 679, 1913.	
	Drummond and Funk, Bioch. J. 8, 598, 1914.	
	Chick and Hume, Med. Res. Com. Rep. 38, p. 29.	6 g. failed, 10 g. sufficed to cure polyneuritic pigeon.
Bran	Chick and Hume, Proc. Roy. Soc. 90B, 44, 1917.	Antineuritic present, probably in aleurone layer, but to less degree than in germ.
Germ	Chick and Hume, Proc. Roy. Soc. 90B, 44, 1917.	Antineuritic of rice grain concentrated in germ.

CEREAL GRAINS AND SEEDS (Continued)

Substance	Reference	Remarks
	Chick and Hume, Med. Res. Com. Rep. 38, p. 29.	0.5 to 1 g. sufficient to cure polyneuritic pigeon.
Rye	Holst, J. Hyg. 7, 629, 1907.	Antineuritic present.
	Osborne and Mendel, J. Biol. Chem. 41, 275, 1920.	40% in diet appears to furnish sufficient *B* for growth.
Seeds of plants, cereals, and pulses	Chick and Hume, J. Roy. Army Med. Corps, 29, 121, 1917.	Principal sources of antineuritic.
	McCollum, Simmonds, and Pitz, J. Biol. Chem. 30, 13, 1917.	Growth-promoting *B* abundant.
Tomato-seed press-cake	Finks and Johns, Am. J. Physiol. 56, 404, 1921.	Can serve as sole source of *B*.
Wheat, kernel	Chick and Hume, Proc. Roy. Soc. 90B, 44, 1917.	Antineuritic concentrated in germ and present to less degree in bran, probably chiefly in aleurone layer.
	McCollum, Simmonds, and Pitz, J. Biol. Chem. 28, 211, 1916-17.	15% in diet furnished sufficient *B* for growth and almost normal reproduction, but not sufficient to permit young to reach weaning age.
	Osborne and Mendel, *Ib*. 41, 275, 1920.	51% in diet appeared to furnish adequate amount of *B* for growth.
Endosperm	Osborne and Mendel, *Ib*. 37, 557, 1919.	Rich in growth-promoting *B*.
	Voegtlin, Lake, and Myers, *Ib*. 41, x, 1920.	Relatively poor in antineuritic.
	Funk and Dubin, *Ib*. 44, 487, 1920.	*B* present (yeast test).
Degerminated	Voegtlin and Myers, *Ib*. 41, x, 1920.	
Degerminated carefully by hand	Osborne and Mendel, 37, 557, 1919.	As efficient as whole wheat in inducing growth.
Germ	McCollum and Davis, J. Biol. Chem. 23, 181, 1915.	Hot water extract of 5 g. wheat germ per 100 g. of ration induced vigorous growth.
	McCollum, Simmonds, and Pitz, *Ib*. 25, 105, 1916.	2% in diet furnishes sufficient *B* for growth.
	Chick and Hume, Proc. Roy. Soc. 90B, 44, 1917.	3 g. every second day prevents polyneuritis in pigeons, but 2 to 3 g. daily produced great increase in body weight, health, and vitality.
Commercial	Osborne and Mendel, J. Biol. Chem. 37, 557, 1919.	Rich in growth-promoting *B*.
Carefully separated by hand	Osborne and Mendel, J. Biol. Chem. 37, 557, 1919.	155 mg. per day suffices to maintain young rats, but will not induce growth.
	Funk and Dubin, *Ib*. 44, 487, 1920.	Ten times as potent as endosperm (yeast test).

Alc. ext.	Voegtlin and Myers, J. Biol. Chem. 41, x, 1920.	Relieves symptoms of polyneuritis.
Alc. ext.	Williams, *Ib*. 38, 473, 1919.	Considerable *B* (yeast test).
Wheat, bran	Sullivan and Voegtlin, J. Biol. Chem. 24, xvi, 1915-16.	Prepared concentrated extract of antineuritic *B* from wheat bran.
	Chick and Hume, Med. Res. Com. Rep. 38, p. 28.	1.5 g. daily gave no protection against polyneuritis. 2.5 g. daily delayed onset of disease about 2 weeks.
Commercial	Osborne and Mendel, J. Biol. Chem. 37, 557, 1919.	5% in diet insufficient for growth of young or maintenance of adult rats, 55% appeared to be sufficient. Much less efficient than commercial germ.
Flour		
Whole wheat	Simpson and Edie, Ann. Trop. Med. Parasil. 5, 321, 1911-12.	Antineuritic present.
	Wellman and Bass, Am. J. Trop. Dis. Prev. Med. 1913.	Antineuritic present in "whole wheat" but lacking in patent flour.
	Voegtlin, Lake, and Myers, U. S. Pub. Health Rep. 33, 647, 1918.	Exclusive diet protects against polyneuritis.
Whole wheat bread	Ohler, J. Med. Res. 31, 239.	Antineuritic present.
Graham	Voegtlin, Lake, and Myers, l. c.	Exclusive diet protected against polyneuritis.
Flour from buhr mill	Voegtlin, Lake, and Myers, l. c.	Less antineuritic than whole wheat but more than patent flour.
Patent flour	Holst, J. Hyg. 7, 629, 1907.	Deficient in antineuritic.
	Simpson and Edie, Ann. Trop. Med. Paras. 5, 321, 1911-12.	Deficient in antineuritic.
	Ohler, l. c.	Deficient in antineuritic.
	Chick and Hume, J. Roy. Army Med. Corps, 29, 121, 1917.	Deficient in antineuritic.
	Voegtlin, Lake, and Myers, l. c.	No protection against polyneuritis.
	Osborne and Mendel, l. c.	Much poorer than commercial germ in growth-promoting power.

TUBERS AND ROOTS

Beets	Osborne and Mendel, J. Biol. Chem. 39, 29, 1919; 41, 451, 1920.	Less growth-promoting power than other common roots.
		Considerable quantities of *B* present in stem and leaves.
	Williams, *Ib*. 46, 113, 1921.	Poor in *B* (yeast test).

TUBERS AND ROOTS (Continued)

Substance	Reference	Remarks
Sugar	Steenbock and Gross, *Ib.* 40, 501, 1919.	25% in diet furnished insufficient amount of *B* for growth.
Carrots	Chick and Hume, Tr. Soc. Med. Hyg. 10, 141, 1916-17.	Rather poor as antineuritic. About equivalent to fresh meat.
	Suguira and Benedict, J. Biol. Chem. 36, 171, 191, 1918.	Ag. and alc. extracts as well as whole carrots cure polyneuritis in cases where the disease has developed quickly (in about 20 days) but were ineffective where the symptoms appeared more slowly.
	Steenbock and Gross, *Ib.* 40, 501, 1919.	15% in ration gave sufficient *B* to permit good growth.
	Osborne and Mendel, *Ib.* 41, 451, 1920.	Comparable to spinach.
	Davis, J. Home Ec. 12, 209, 1920.	5 g. per day gave no protection against polyneuritis.
	Williams, J. Biol. Chem. 46, 113, 1921.	About one-fifth value of brewers' yeast (yeast test).
Canned	Davis, J. Home Ec. 12, 209, 1920.	10 g. per day gave some protection against polyneuritis.
Alc ext.	Zilva, Bioch. J. 14, 494, 1920.	Antineuritic present.
Dasheen	Steenbock and Gross, l. c.	15% in diet supplied sufficient *B* for growth.
Mangel	Steenbock and Gross, l. c.	25% in diet did not supply sufficient *B* for growth.
Onion	Chamberlain, Vedder and Williams, Phil. J. Sci. 7B, 45, 1912.	Ag. ext. without effect on polyneuritic pigeons.
	Chick and Hume, Tr. Soc. Med. Hyg. 10, 141, 1916-17.	Rather poor as antineuritic. About equivalent to fresh meat.
	Davis, l. c.	10 g. per day equivalent to 8 g. boiled or baked potato as antineuritic.
	Osborne and Mendel, J. Biol. Chem. 39, 29, 1920.	Considerable growth-promoting *B* present.
Potato (raw or cooked)	Vedder and Clark, Phil. J. Sci. 7B, 6, 1912.	10 g. per day gave protection against polyneuritis to 2 out of 4 fowls on a polished rice diet.
Juice	Vedder and Clark, Phil. J. Sci. 7B, 6, 1912.	No protection against polyneuritis.
	McCollum and Kennedy, J. Biol. Chem. 24, 491, 1915-16.	12 c. c. daily brought about gradual improvement in polyneuritic bird.
Peel	Chick and Hume, Med. Res. Com. Rep. 38, p. 30.	Alc. extract equivalent to 630 g. fresh peelings brought about temporary improvement but failed to cure polyneuritic pigeon.
Peeled	Chick and Hume, Med. Res. Com. Rep. 38, p. 30.	Alc. extract equivalent to 350 g. of fresh material cured a polyneuritic pigeon.

Cooked	McCollum, Simmonds, and Parsons, J. Biol. Chem. 36, 197, 1918.	84.5% in diet furnished enough *B* for growth of young rats.
Peeled, dried	Osborne and Mendel, J. Biol. Chem. 41, 451, 1920.	Comparable to turnip, carrot, etc. 2 g. daily did not promote normal growth.
Peel	Osborne and Mendel, J. Biol. Chem. 41, 451, 1920.	No richer in growth-promoting *B* than corresponding quantities of whole potato.
Raw	Davis, J. Home Ec. 12, 209, 1920.	8 g. per day protected pigeons against polyneuritis for 590 days.
Cooked	Davis, J. Home Ec. 12, 209, 1920.	Not equivalent to raw potato in antineuritic power.
Dried (com.)	Davis, J. Home Ec. 12, 209, 1920.	2 g. per day gave no protection, 4 g. at least partial protection against polyneuritis.
Dried	Williams, J. Biol. Chem. 46, 113, 1921.	Less than one-third value of brewers' yeast. Equivalent to cabbage (yeast test).
Turnip, dried	Osborne and Mendel, J. Biol. Chem. 39, 29, 1919; 41, 451, 1920.	Considerable quantities of growth-promoting *B*. Comparable to spinach.
	Williams, *Ib*. 46, 113, 1920.	About one-sixth value of brewers' yeast (yeast test).
Rutabagas	Steenbock and Gross, *Ib*. 40, 501, 1919.	15% in diet sufficient to induce normal growth.
FRUITS		
Apples	Osborne and Mendel, J. Biol. Chem. 42, 465, 1920.	Some, but not much, growth-promoting *B*.
Banana	Loeb and Northrop, *Ib*. 27, 309, 1916.	Probably deficient in growth-promoting *B*.
	Suguira and Benedict, *Ib*. 36, 171, 1918.	Deficient in growth-promoting *B*.
Currants, dried	Chick and Hume, Med. Res. Com. Rep. 38, p. 30.	Alcoholic extract apparently devoid of antineuritic power.
Dates, dried	Chick and Hume, Med. Res. Com. Rep. 38, p. 30.	Alcoholic extract corresponding to 26 g. of fruit brought about slight temporary improvement in polyneuritic bird.
Grape juice, commercial	Davis, J. Home Ec. 12, 209, 1920.	3 g. daily gave no protection against polyneuritis.
	Osborne and Mendel, l. c.	Far less efficient than orange juice as a growth stimulant.
Grapefruit juice	Osborne and Mendel, l. c.	Growth-promoting properties about equivalent to orange juice or milk.
Lemon juice	Osborne and Mendel, l. c.	10 c. c. per day insufficient for growing rats. About equivalent to orange juice in growth-promoting power.

FRUITS (Continued)

Substance	Reference	Remarks
Lime juice	Funk, Bioch. J. 7, 81, 1913.	Antineuritic present.
Orange juice	Harden and Zilva, *Ib*. 12, 93, 1918.	Antineuritic present in very small amounts.
	Byfield, Daniels and Loughlin, Am. J. Dis. Child. 19, 349, 1920.	75 c. c. orange juice per 100 g. of food promotes maximal growth, 55 c. c. does not.
	Osborne and Mendel, J. Biol. Chem. 42, 465, 1920.	Fresh juice about equivalent to milk in growth-promoting power.
Peel	Osborne and Mendel, J. Biol. Chem. 42, 465, 1920.	5 g. per day efficacious as growth stimulant.
Pears	Osborne and Mendel, J. Biol. Chem. 42, 465, 1920.	Some, but not much, growth-promoting power.
Prunes	Osborne and Mendel, J. Biol. Chem. 42, 465, 1920.	Contain more growth-promoting *B* than apples or pears, though probably not very rich.
Raisins	Davis, J. Home Ec. 12, 209, 1920.	3 g. per day insufficient, 8 g. sufficient to give protection against polyneuritis.
Tomato	Osborne and Mendel, J. Biol. Chem. 39, 29, 1919.	Rich in growth-promoting *B*.
Canned	Osborne and Mendel, J. Biol. Chem. 39, 29, 1919.	1 g. daily furnished enough *B* to promote good growth, even 0.2 g. occasionally produced limited growth or, at least, maintenance.
	Williams, *Ib*. 46, 113, 1921.	Less than one-sixth the value of brewers' yeast (yeast test).
Nuts		
Almond	Cajori, J. Biol. Chem. 43, 583, 1920.	Normal growth when *B* was supplied by 2 g. of almonds daily. 1 g. less effective than 0.5 g. of pecan or chestnut.
Brazil nut		1 g. induces normal growth, 0.5 g. permits slow growth.
Chestnut		1 g. induces normal growth, 0.5 g. permits slow growth.
Coconut presscake	Johns, Finks, and Paul, *Ib*. 37, 497, 1919.	Induced satisfactory growth as sole source of *B*.
Filberts	Cajori, l. c.	Equivalent to almond.
Hickory	Cajori, l. c.	1 g. daily induced renewed growth in animals declining for lack of *B*.

NUTS AND MISCELLANEOUS

Peanut	Vedder and Clark, Phil. J. Sci. 7B, 1, 1912.	10 g. per day protects fowls on polished rice diet against polyneuritis for at least 60 days.
	Daniels and Loughlin, J. Biol. Chem. 33, 295, 1918.	56% in ration gave sufficient *B* for normal growth of rats.
	Grieg, 2nd J. Med. Res. 6, 143, 1918.	Rich in antineuritic.
Pecan	Cajori, J. Biol. Chem. 43, 583, 1920.	1 g. gives normal growth, 0.5 g. permits of partial recovery and slow growth in animals which have declined for lack of *B*.
Pine-nut	Cajori, J. Biol. Chem. 43, 583, 1920.	1 g. daily induced recovery of animals which had declined for lack of *B*.
Walnuts, black	Cajori, J. Biol. Chem. 43, 583, 1920.	Equivalent to chestnuts.
English	Cajori, J. Biol. Chem. 43, 583, 1920.	2 g. daily gives normal growth.

MISCELLANEOUS

Beer	Holst, Centr. f. Bakt. Mar. 27, 1918.	Useful as curative substance in treatment of beriberi.
Coffee (roasted)	Di Mattei, Policlin. 27, 1011, 1920.	Polyneuritic pigeons cured temporarily but not permanently by coffee infusion. Probably not true vitamin present.
Crisco (autoclaved for 3 hours)	Funk and Dubin, J. Biol. Chem. 44, 487, 1920.	No *B* present (yeast test).
Honey	Dutcher, *Ib*. 36, 551, 1918.	Traces of antineuritic present (possibly due to pollen grains).
Malt extract	Hawk, Smith and Bergeim, Am. J. Physiol. 55, 339, 1921.	Not more than minimal amount of *B* in strained honey.
	Cooper, J. Hyg. 14, 12, 1914.	Two samples very efficient as antineuritic, while third was inactive.
	Van der Wielen, Pharm. Weekblad. 52, 673, 1915.	Can be used for preparation of antineuritic extract.
Nectar	Dutcher, l. c.	No antineuritic present.
Pollen (corn)	Dutcher, l. c.	Relatively rich in antineuritic.
Yeast, autolyzed	Chamberlain and Vedder, Phil. J. Sci. 6B, 395, 1911.	Antineuritic present.
	Cooper and Funk, Lanc. 1911, ii, 1266.	
	Edie, Evans, Moore, Simpson and Webster, Bioch. J. 6, 234, 1912.	
	Cooper, J. Hyg. 12, 436; 14, 12, 1914.	

MISCELLANEOUS (Continued)

Substance	Reference	Remarks
	Funk, Bioch. Bull. 5, 1, 1916.	Richer in antineuritic than any known foodstuff.
	Seidell, J. Biol. Chem. 29, 145, 1917.	0.5 to 1 c. c. of filtrate just sufficient to replace deficiency of polished rice.
	Voegtlin, Lake, and Myers, U. S. Pub. Health Rep. 33, 647, 1918.	
	Chick and Hume, Med. Res. Com. Rep. 38, p. 30.	2 g. induced slow cure in polyneuritic pigeon.
	Holst, J. Hyg. 7, 629, 1907.	Delays symptoms of polyneuritis, but not loss of weight.
Fresh	Hopkins, J. Physiol. 44, 425, 1912.	Good source of antineuritic.
	Funk, J. Biol. Chem. 27, 1, 1916.	
	Funk and Macallum, *Ib*. 23, 413, 1915; 27, 51, 1916.	
	Osborne and Mendel, *Ib*. 31, 149, 1917; 41, 451, 1920.	Richest of all substances tested in growth-promoting power.
	Seidell, *Ib*. 29, 145, 1917.	Less effective than autolyzed.
Extract		
Commercial	Chick and Hume, Med. Res. Com. Rep. 38. p. 30.	1.5 to 2 g. effected complete cure in polyneuritic bird.
	Voegtlin, Lake, and Myers, U. S. Pub. Health Rep. 33, 647, 1918.	Addition to bread in amounts used by bakers does not prevent polyneuritis but retards onset slightly.
Extract, brewers'	Williams, J. Biol. Chem. 46, 113, 1921.	Very rich (yeast test).
Marmite, commercial preparation of yeast	Willcox, Lanc. 1919, ii, 979.	Used as prophylactic in beriberi.

MEAT AND FISH

Substance	Reference	Remarks
Beef, dried	Pitz, J. Biol. Chem. 36, 439, 1918.	5% in diet of rolled oats with milk ad lib. did not delay onset of scurvy, but prolonged life of animal. 10% delayed onset of disease.
	Givens and McClugage, Sci. 51, 273, 1920.	No appreciable antiscorbutic present.
Fresh raw	Dutcher, Pierson, and Biester, J. Biol. Chem. 42, 301, 1920.	Lacking in *C*.
Frozen	Wiltshire, Lanc. 1918, ii, 811.	Poor in *C*.
	Comrie, Edin. Med. J. 24, 207, 1920.	Scurvy developed on a diet including seven and one-quarter oz. per day of frozen or tinned meat or salt herring.

Fish muscle	Parsons, J. Biol. Chem. 44, 589, 1920.	C absent or very low.
Salted salmon	Koga, Bul. Naval Med. Ass. Tokyo, 1920, p. 2.	No protection.
Liver, beef	Parsons, J. Biol. Chem. 44, 589, 1920.	High in C.
Pig	Parsons, J. Biol. Chem. 44, 589, 1920.	High in C.
Rat	Parsons, J. Biol. Chem. 44, 589, 1920.	High in C.
Seal	Holst, 15th Int. Cong. Hyg. Wash. 1912, ii, 588.	Used by Eskimos as remedy for scurvy.
Cod oil	Cohen and Mendel, J. Biol. Chem. 35, 443, 1918.	1 c. c. daily gave no protection against scurvy.
	Hess and Unger, *Ib.* 35, 479, 1918.	0.5 to 1 c. c. gave no protection to guinea pigs, nor is it effective in infantile scurvy.
Meat, fresh raw	Barlow, Lanc. 1894, ii, 1075.	Less antiscorbutic than fresh fruit or vegetables.
	Willcox, Lanc. 1917, ii, 677; 1919, ii, 979.	Valuable antiscorbutic.
	Stevenson, J. Roy. Army Med. Corps, 35, 218, 1920.	Distinct antiscorbutic value.
(Rat muscle)	Parsons, l. c.	Very little, if any, C.
Salt pork	Koga, l. c.	No protection.
Juice, raw	Chick, Hume, and Skelton, Lanc. 1919, ii, 322.	20 c. c. per day gave slight protection against scurvy in some cases.
Cooked	Barlow, Lanc. 1894, ii, 1075.	Recommended as antiscorbutic for infants.
Extract	Dutcher, Pierson, and Biester, J. Biol. Chem. 42, 301, 1920.	No demonstrable antiscorbutic present.
MILK		
Milk, fresh	Curran, Dub. J. Med. Sci. 7, 83, 1847.	1 pt. of milk daily insufficient to protect adults from scurvy on diet deficient in fresh meat and vegetables.
	Barlow, Lanc. 1894, ii, 1075.	Less effective antiscorbutic than fresh vegetables or fruits.
	Funk, J. Biol. Chem. 25, 409, 1916.	50 c. c. daily gave protection to guinea pigs on a diet of oats.
	Jackson and Moore, J. Infect. Dis. 19, 478, 1916.	Milk fed *ad lib.* afforded no protection to 36 out of 41 guinea pigs.
	Chick, Hume, and Skelton, Bioch. J. 12, 131, 1918; Lanc. 1918, i, 1.	85 to 135 c. c. daily gave complete protection against scurvy for about three months; on 30 to 50 c. c. death from scurvy occurred in about 30 days.
	Cohen and Mendel, J. Biol. Chem. 35, 438, 1918.	Small quantities of milk ineffective, large amounts gave practically complete protection against scurvey.

MILK (Continued)

Substance	Reference	Remarks
	Drummond, Lanc. 1918, ii, 482.	Little antiscorbutic present.
	Barnes and Hume, Bioch. J. 13, 306, 1919.	Guinea pigs on diet of oats and bran require 100 to 150 c. c. for protection; monkeys of 2 to 3 kg. body weight require 125 to 175 c. c.
	Hart, Steenbock, and Smith, J. Biol. Chem. 38, 305, 1919.	47 c. c. per day added to rolled oats diet will not prevent scurvy in guinea pigs; 84 c. c. per day added to diet of oats and hay gives absolute protection.
	Hess and Unger, *Ib.* 38, 293, 1919.	80 c. c. daily cured scurvy.
Sour	Comrie, Edin. Med. J. 24, 207, 1920.	More effective than lemon juice for treatment of human scurvy.
	Stevenson, J. Roy. Army Med. Corps, 35, 218, 1920.	Sour milk and lemon juice added to ordinary diet best cure for human scurvy.
Condensed	Davis, J. Home Ec. 12, 209, 1920.	75 c. c. per day allowed onset of scurvy in 4 weeks.
	Hart, Steenbock, and Smith, J. Biol. Chem. 38, 305, 1919.	36 to 52 c. c. per day allowed onset of scurvy in 30 to 40 days.
Milk, dried (Just-Hatmaker process)	Barnes and Hume, Bioch. J. 13, 306, 1919.	Equivalent of 250 to 300 c. c. fluid milk required to give protection afforded by 150 to 175 c. c. of fresh milk.
	Hart, Steenbock, and Smith, J. Biol. Chem. 38, 293, 1919.	Little antiscorbutic value in some specimens at least. Equivalent of 40 to 45 c. c. fluid milk allowed development of scurvy in 5 to 6 weeks; on 75 to 90 c. c. onset retarded for 5 to 15 weeks.
(Just-Hatmaker)	Hess and Unger, J. Biol. Chem. 38, 293, 1919.	Little less efficient than fresh milk. Cures infantile scurvy.
	Hopkins, Lanc. 1919, ii, 979.	Infants do not develop scurvy on a diet of dried milk.
(Just-Hatmaker)	Davis, J. Home Ec. 12, 209, 1920.	Equivalent of 150 c. c. fluid milk gave complete protection to guinea pigs.
Butter	Cohen and Mendel, J. Biol. Chem. 35, 432, 1918.	No antiscorbutic demonstrable.

MISCELLANEOUS

Substance	Reference	Remarks
Egg, whole	Hess and Unger, J. Biol. Chem. 35, 479, 1918.	Failed to delay onset of scurvy in guinea pigs, nor was it effective in treatment of infantile scurvy.

LEAFY VEGETABLES AND GRASSES

Cabbage, dried at high temperature	Holst and Frölich, Ztschr. Hyg. 72, 1, 1912.	No antiscorbutic present.
Dried at 37°	Holst and Frölich, Ztschr. Hyg. 72, 1, 1912.	Slight antiscorbutic value after 6 mos. storage in incubator.
	Chick and Rhodes, Lanc. 1918, ii, 774.	2 g. per day protected guinea pigs.
	Cohen and Mendel, J. Biol. Chem. 35, 425, 1918.	10 g. per day protected guinea pigs on soy bean cracker diet, 35 g. per day added to diet of oats permits successful reproduction.
Dried at 70 to 80°	Cohen and Mendel, J. Biol. Chem. 35, 425, 1918.	1 g. and 80 c. c. of milk daily maintained guinea pig in excellent condition, 3 g. per day cured scurvy that had developed on soy bean cracker diet.
Raw	Givens and Cohen, *Ib.* 36, 127, 1918.	10 g. per day gives complete protection to guinea pigs on soy bean diet, also initiates prompt recovery provided disease has not gone too far.
Dried at 65 to 78°	Givens and Cohen, *Ib.* 36, 127, 1918.	1 to 2 g. per day gives no protection.
Dried at 65 to 78° and cooked	Givens and Cohen, *Ib.* 36, 127, 1918.	Equivalent of 10 g. raw gave no protection.
Dried at 42 to 52°	Givens and Cohen, *Ib.* 36, 127, 1918.	1 g. per day delayed onset of disease and prolonged life.
Fresh	Chick, Hume, and Skelton, Lanc. 1919, ii, 322.	1.5 g. per day minimum dose affording protection.
	Delf. quoted by Campbell and Chick, *Ib.* 321.	0.5 g. per day partial protection, 1.5 g. complete protection on diet of oats, bran, and autoclaved milk.
Fresh cooked 60′ at 90°	Delf. quoted by Campbell and Chick, *Ib.* 321.	5 g. per day gave no protection.
Cooked 20′ at 100°	Delf. quoted by Campbell and Chick, *Ib.* 321.	5 g. per day gave almost complete protection.
Canned, 60′ at 90 to 100°	Delf. quoted by Campbell and Chick, *Ib.* 321.	5 g. per day gave partial protection in 2 out of 3 cases, 7.5 g. per day gave complete protection.
Fresh	Davis, priv. com. 1920.	7 g. per day kept guinea pigs in good condition for 385 days. On 5 g. they lost weight, which was regained when they were returned to the 7 g. ration.
Dandelions	Holst and Frölich, Ztsch. Hyg. 72, 1, 1912; 75, 334, 19B.	Antiscorbutic when fresh, but lose power completely on drying.

LEAFY VEGETABLES AND GRASSES (Continued)

Substance	Reference	Remarks
Hay	Hess and Unger, Proc. Soc. Exp. Biol. Med. 15, 82, 1918.	Valuable as roughage, but did not prevent scurvy when added to oats and water diet.
	Cohen and Mendel, J. Biol. Chem. 35, 432, 1918.	No antiscorbutic present.
	Barnes and Hume, Bioch. J. 13, 306, 1919.	Probably not devoid of antiscorbutic, amount varying with age of hay.
	Hart, Steenbock, and Smith, J. Biol. Chem. 38, 305, 1919.	Less milk required for protection of guinea pigs against scurvy when hay forms part of diet.
Rhubarb	Curran, Dublin J. Med. Sci. 1847, 7, 83.	Possesses antiscorbutic power.
	Pierson and Dutcher, Sci. 51, 70, 1920.	Guinea pig scurvy cured by feeding rhubarb, raw or after 15 minutes cooking.
Spinach, canned	Davis, priv. com. 1920.	Great variation in different lots of commercial canned spinach. Complete protection in some cases, poor in others.

CEREAL GRAINS AND SEEDS

Substance	Reference	Remarks
Barley, whole	Cohen and Mendel, J. Biol. Chem. 35, 425, 1918.	Scurvy develops rapidly on exclusive diet of barley.
Germinated	Weill, Cluzet, and Mouriquand, C. r. Soc. Biol. 70, 36, 1917.	Better than ungerminated grain as complete food for guinea pigs.
	Cohen and Mendel, l. c.	Scurvy prevented for some time on exclusive diet of germinated barley.
Beans, dried	Davis, priv. com. 1920.	5 g. daily gave slight protection.
Germinated	Wiltshire, Lanc. 1918, ii, 811.	More efficient than lemon juice.
	Comrie, Edin. Med. J. 1920, 207.	Less effective than germinated peas.
	Stevenson, J. Roy. Army Med. Corps, 35, 218, 1920.	Useful for prevention but not for cure owing to their hard consistency.
Red, germinated	Koga, Bul. Naval Med. Ass. Tokyo, 1920, p. 2.	20 g. per day gave protection to guinea pigs for 41 days.
String, fresh raw	Campbell and Chick, Lanc. 1919, ii, 321.	5 g. per day afforded no protection.
Cooked 140′ at 100°	Campbell and Chick, Lanc. 1919, ii, 321.	20 g. per day afforded no protection.
	Chick, Hume, and Skelton, *Ib.* 1919, ii, 774.	5 g. per day minimum dose giving protection.
	Davis, priv. com. 1920.	Great variation in different specimens. Litter of guinea pigs successfully raised on 30 g. per day.
String, canned	Davis, priv. com. 1920.	15 g. daily delayed but did not prevent onset of scurvy.

Soy flour		
Heated at 120° for 30′	Cohen and Mendel, J. Biol. Chem. 35, 435, 1918.	No antiscorbutic present.
Cereals, dry	Fürst, Z. Hyg. Infekt. 72, 121, 1912.	Antiscorbutic power lacking, but present after germination.
	Chick and Hume, J. Roy. Army Med. Corps, 29, 121, 1917.	No *C* present.
Germinated	Chick and Hume, J. Roy. Army Med. Corps, 29, 121, 1917.	Antiscorbutic power appears with beginning of active cell life.
	Weill, Mouriquand, and Peronnet, C. r. Soc. Biol. June 8th, 1918.	Question the efficacy of sprouted cereals as antiscorbutic.
	McClendon, Cole, Engstrand and Middlekauf, J. Biol. Chem. 40, 243, 1919.	Rich in antiscorbutic, especially when acrospire projects 0.5 in beyond grain.
Corn, (see maize)		
Maize, germinated	Fürst, Nord. Med. Arch. Abt. ii, 1910. Supp. 349.	Fresh sprouted corn antiscorbutic, but dried corn, whether germinated or not, without protective power.
Oats, rolled	Cohen and Mendel, J. Biol. Chem. 35, 425, 1918.	Exclusive diet of oats produces rapid development of scurvy.
Germinated	Cohen and Mendel, J. Biol. Chem. 35, 425, 1918.	Exclusive diet maintained guinea pig without development of scurvy.
	Fürst, l. c.	Sprouted oats valuable as antiscorbutic.
Peas, germinated	Chick, Hume, and Skelton, Lanc. 1919, ii, 774.	5 g. per day minimum dose affording protection.
	Comrie, Edin. Med. J. 207, 1920.	More effective than germinated beans.
Pulses (lentils, peas, and beans)	Fürst, Z. Hyg. Infekt. 72, 121, 1912.	Lack antiscorbutic power when dry, but acquire it after 2 to 3 days germination.
Germinated	Chick and Rhodes, J. Roy. Army Med. Corps, 29, 121, 1917.	Valuable and convenient antiscorbutic.
	Chick and Delf, Bioch. J. 13, 199, 1919.	Equivalent to green beans or potatoes. Antiscorbutic power increased 5 to 6 fold on germinating 48 hours.
Seeds, dry	Davis, priv. com. 1920.	Ration of Kaffir corn, split peas, barley, corn, oats, and hay delayed onset of scurvy in guinea pigs about 20 days. Rabbits continued in good condition on this ration for 300 days, but reproduction was not successful.
Wheat embryo	Hess, Am. J. Dis. Child. 13, 98, 1917.	Antiscorbutic value very doubtful.

TUBERS AND ROOTS

Substance	Reference	Remarks
Beet root	Chick and Rhodes, Lanc. 1918, ii, 774.	Rather poor in *C*.
Juice	Chick, Hume, and Skelton, *Ib*. 1919, ii, 774.	20 c. c. per day gives slight, but not complete protection.
Carrot juice	Holst and Frölich, Z. Hyg. 72, 1, 1911.	Antiscorbutic present.
	Chick and Rhodes, l. c.	20 c. c. equivalent in antiscorbutic power to 2.5 c. c. swede juice.
	Chick, Hume, and Skelton.	20 c. c. minimum dose giving protection.
Alc. ext.	Zilva, Bioch. J. 14, 494, 1920.	Antiscorbutic present.
Onion, raw	Munson, Theory and Practice of Mil. Hyg. N. Y. 1901.	Especially valuable prophylactic against scurvy.
Boiled	Davis, priv. com. 1920.	20 g. per day failed to protect, although one guinea pig was successfully reared to the age of 120 days with onion as sole source of *C*.
Potato, raw	Munson, l. c.	Valuable prophylactic against scurvy.
	Givens and McClugage, J. Biol. Chem. 42, 491, 1920.	10 g. daily protects guinea pigs on soy bean diet for at least 6 times as long as soy bean alone.
	Bezssonoff, C. r. 172, 92, 1921.	Equal to cabbage or dandelion. (If potatoes are crushed action is much inferior.)
Juice	Funk, J. Biol. Chem. 25, 409, 1916.	25 c. c. daily failed to protect.
	Bezssonoff, l. c.	Antiscorbutic present, but less than in equivalent weight of uncrushed potato (residue contains practically no antiscorbutic).
Cooked ½ hour in salt water	Holst and Frölich, Z. Hyg. 72, 1, 1912.	Exclusive diet gave complete protection.
Cooked	Hess and Fish, Am. J. Dis. Child. 8, 386, 1914.	May be used as antiscorbutic for children.
Steamed	Chick and Rhodes, Lanc. 1918, ii, 774.	20 g. per day effective antiscorbutic.
Cooked ½ hour at 100°	Chick, Hume, and Skelton, Lanc. 1919, ii, 774.	20 g. per day minimum dose affording protection.
Boiled	Davis, priv. com. 1920.	8 g. per day gives sufficient *C* for successful growth, but not more than 20 g. required for satisfactory reproduction. 5 g. gives partial, but not complete protection against scurvy.
	Givens and McClugage, l. c.	Cooking for 15 minutes at 100° does not appreciably reduce antiscorbutic power, cooking for an hour does.

Dried (commercial)	Holst and Frölich, Z. Hyg. 72, 1, 1912.	No protection.
Dried at 37°		No protection.
Dried at 30° in vac.		No protection.
Dried at 65 to 70° and cooked	Givens and Cohen, J. Biol. Chem. 36, 127, 1918.	Equivalent of 5 g. raw per day gave no protection.
	Givens and McClugage, l. c.	Results vary greatly with temperature and duration of drying. 55 to 60° being apparently optimum temperature. 2.5 g. dried at this temperature gives partial protection.
Radish, pickled	Koga, Bul. Naval Med. Ass. Tokyo, 1920. p. 2.	No protection.
Turnip, swede	Chick and Rhodes, Lanc. 1918, ii, 774.	2.5 c. c. of expressed juice per day sufficient to protect guinea pigs against scurvy.
	Chick, Hume and Skelton, *Ib.* 1919, ii, 774.	
Dehydrated vegetables	Holst and Frölich, Z. Hyg. 72, 1, 1912; 75, 334, 1913.	Little or no antiscorbutic present.
	Chick and Hume, Tr. Soc. Trop. Med. Hyg. 10, 141, 1917.	All dried foodstuffs examined, including desiccated vegetables, more or less deficient in antiscorbutic power.
	Hess and Unger, J. Biol. Chem. 35, 479, 1918.	Little or no antiscorbutic power.
FRUITS		
Apple, dried	Davis, priv. com. 1920.	Affords some protection.
Banana	Suguira and Benedict, J. Biol. Chem. 36, 171, 1918.	Scurvy developed in some cases in rats fed exclusively on banana.
	Lewis, *Ib.* 40, 91, 1919.	25 g. per day added to diet of rolled oats protects against scurvy, smaller amounts insufficient.
Grape juice	Chick and Rhodes, Lanc. 1918, ii, 774.	About one-tenth as efficient as orange juice.
Dried	Givens and Macy, J. Biol. Chem. 46, xi, 1921.	*C* absent.
Grapefruit juice, dried	Givens and Macy, J. Biol. Chem. 46, xi, 1921.	Significant amount of *C* present.
Lemon juice, fresh	Chick, Hume, and Skelton, Lanc. 1919, ii, 322.	1.5 c. c. per day minimum dose giving protection.
Dried	Givens and Macy, l. c.	Significant amount of *C* present.
Dried in vac.	Bassett-Smith, Lanc. 1920, i, 1102.	Satisfactory prophylactic and cure for earlier stages of disease.

FRUITS (Coninued)

Substance	Reference	Remarks
Freed from citric acid	Harden and Zilva, Bioch. J. 12, 270, 1918.	Powerful antiscorbutic.
	Harden, Zilva, and Still, Lanc. 1919, i, 17.	Powerful antiscorbutic.
Lime juice, fresh	Chick, Hume ,and Skelton, l. c.	10 c. c. per day minimum dose affording protection.
Preserved	Funk, J. Biol. Chem. 25, 415, 1916.	Very poor antiscorbutic.
	Willcox, Lanc. 1919, ii, 979.	Freshly preserved with alcohol and salicylic acid seems to have some value, old none at all.
Orange juice	Chick and Rhodes, Lanc. 1918, ii, 774.	Best of fruits as antiscorbutic, but little if any better than swede juice.
	Cohen and Mendel, J. Biol. Chem. 34, 425, 1918.	5 c. c. per day gives complete protection.
	Hess, Proc. Soc. Exp. Biol. Med. May 15th, 1918.	Intravenous injection effective cure for scurvy.
Dried	Givens and McClugage, Am. J. Dis. Child. 18, 30, 1919.	Useful antiscorbutic.
	Givens and Macy, J. Biol. Chem. 46, xi, 1921.	Retains antiscorbutic potency two years after dehydration.
	McClendon, Bowers, and Sedgwick, *Ib*. 46, ix, 1921.	Equivalent of 1.25 c. c. fresh juice gave complete protection to guinea pigs.
Orange peel	Hess, Am. J. Dis. Child. 12, 152, 1916.	Antiscorbutic present.
	Hess and Unger, J. Biol. Chem. 35, 479, 1918.	Marked antiscorbutic power.
Plums, pickled	Koga, Bul. Naval Med. Ass. Tokyo, 1920, p. 2.	No antiscorbutic power.
Prunes	Hess and Unger, l. c.	Practically no antiscorbutic power.
Raspberry juice, dried	Givens and Macy, l. c.	No antiscorbutic power.
Tomato	Munson, Theory and Practice of Mil. Hyg. N. Y. 1901.	Valuable prophylactic against scurvy.
	Hess and Unger, Proc. Soc. Exp. Biol. Med. 16, 1, 1918.	Good antiscorbutic, raw or canned.
	Hess, N. Y. State J. Med. 20, 209; J. Am. Med. Ass. 75, 568, 1920.	Most serviceable antiscorbutic for infants. Should be fed at the rate of about 30 g. (1 oz.) per day.
	Givens and McClugage, J. Biol. Chem. 37, 253, 1919.	10 g. per day efficient either as preventive or cure for guinea pigs on soy bean diet.
Dried	Givens and McClugage, J. Biol. Chem. 37, 253, 1919.	Retain significant amount of potency, whether dried at high temperature (55 to 60° C) or low temperatures (35 to 40° C).

Canned	Chick and Rhodes, quoted by Barnes and Hume, Bioch. J. 13, 327, 1919.	Confirm results of Hess and Unger as to antiscorbutic value of canned tomatoes.
Tropical Fruits		
"Amchier" (mango preparation)	Chick, Hume, and Skelton, Lanc. 1919, ii, 322.	Slight protection but not complete.
"Anola" (dried fruit of Phyllanthus emblica)	McNab, Quart. J. Calcutta Med. Phys. Soc. 1. 306.	Has antiscorbutic value.
Cocum	Chick, Hume, and Skelton, l. c.	Slight protection in some cases but not complete.
Mango, dried	Charles, Afghan Bound. Com. Rep. 1884.	Has antiscorbutic value.
Tamarind	Chick, Hume, and Skelton, l. c.	3 to 5 g. per day retarded but did not prevent onset of scurvy.
	Willcox, Lanc. 1919, ii, 979.	Some value as antiscorbutic.
Miscellaneous		
Beer	Smith, Lanc. 1917, ii, 813.	Beer used before 1850 apparently had antiscorbutic value; that made from "high-dried kilned malt" in modern process little if any.
	Dyke, Lanc. 1918, ii, 513.	Beer prepared from germinated grain antiscorbutic.
Cider, commercial pasteurized	Davis, priv. com. 1920.	Some, but little antiscorbutic.
Honey	Faber, J. Biol. Chem. 43, 113, 1920.	No demonstrable antiscorbutic.
	Hawk, Smith and Bergeim, Am. J. Physiol. 55, 339, 1921.	No protection afforded to guinea pigs.
Lactose	Cohen and Mendel, *Ib*. 35, 425, 1918.	No demonstrable antiscorbutic.
	Hart, Steenbock, and Smith, *Ib*. 38, 305, 1919.	No demonstrable antiscorbutic.
Lactose and other carbohydrates	Harden and Zilva, Bioch. J. 12, 270, 1918.	No demonstrable antiscorbutic.
Malt soup	Gerstenberger, Am. J. Dis. Child. 21, 315, 1921.	May possess antiscorbutic properties if barley is of proper age and state of germination or possesses an unusually high content of *C*.
Yeast	Hess, Am. J. Dis. Child. 13, 98, 1917.	No antiscorbutic value.
	Cohen and Mendel, J. Biol. Chem. 35, 432, 1918.	No antiscorbutic value demonstrable.

INDEX OF AUTHORS

Abderhalden, 56, 174, 215
Abels, 219, 225
Ackerson, 271
Ackroyd, 56
Agulhon, 318
Alcastre, 118
Alencastre, 116
Allbut, 194
Allemann, 274
Allen, 163, 169, 171, 173, 185, 207, 288
Allesandrini, 247
Amand, 43, 313
Anderson, 103, 218, 279, 284
Andrews, 202, 220, 277, 293
Anrep, 127
Appleton, 241
Armstrong, 186
Arnold, 262
Aulde, 247
Avery, 317

Babcock, 274
Babes, 242
Bacharach, 271, 291
Bachman, 187, 297
Bachmann, 35, 39, 314
Bachstrom, 232
Baly, 230
Bang, 278
Barbe, 206, 209
Barlow, 219, 298, 345
Barnes, 86, 104, 270, 277, 283, 284, 290, 298, 346, 348, 353
Barnett, 92
Barr, 309
Barton, 293, 298
Basch, 193
Bass, 205, 339
Bassett-Smith, 103, 108, 144, 351
Beebe, 181
Beger, 56
Behr, 273
Benedict, 82, 98, 131, 151, 161, 309, 329, 340, 341, 351
Bergeim, 157, 305, 343, 353
Bergheim, 148, 181, 329
Bezssonoff, 135, 350
Bidault, 103
Bierman, 182
Bierry, 180
Biester, 86, 88, 344, 345
Bigland, 169, 170, 258
Bland-Sutton, 194
Bloch, 235, 296
Bloombergh, 205
Blosser, 247
Bogel, 173, 183
Bolton, 71
Bonar, 199
Bordoni, 247
Bory, 260
Bosshard, 137
Bosworth, 263, 297
Boutwell, 34, 63, 67, 70, 75, 76, 91, 92, 94, 111, 112, 136, 237, 238, 307, 326, 328
Bowers, 144, 352
Boyd, 244, 249, 257
Braddon, 31, 95, 180, 205, 210
Brandt, 218
Bravetta, 256
Breinl, 209
Brennemann, 280
Brewster, 32
Brill, 96, 116, 118
Buckner, 56
Buell, 67, 69, 75, 76, 92, 94, 113
Bulley, 237, 238
Byfield, 296, 342

Cajori, 342, 343
Campbell, 33, 85, 93, 94, 99, 100, 102, 134, 228, 348
Carbone, 247
Cartier, 217

Caspari, 15, 210
Cave, 165
Cazzamalli, 247
Cessna, 43, 314
Chamberlain, 115, 117, 132, 150, 205, 214, 337, 340, 343
Charles, 353
Cheadle, 194
Chick, 18, 34, 78, 82, 83, 84, 85, 88, 89, 94, 95, 96, 98, 99, 100, 102, 150, 151, 152, 155, 156, 164, 221, 232, 237, 254, 257, 259, 269, 284, 288, 298, 306, 307, 309, 326, 327, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 344, 345, 347, 348, 349, 350, 351, 352, 353
Chittenden, 253, 256, 257
Clair, 210
Clark, 151, 205, 207, 213, 234, 238, 336, 340, 343
Clarke, 311
Clementi, 253, 257
Clement Lucas, 195
Cluzet, 348
Cohen, 25, 100, 106, 158, 221, 222, 226, 345, 346, 347, 348, 349, 351, 352, 353
Cole, 100, 220, 223, 317, 349
Comby, 219
Comrie, 88, 270, 344, 346, 348, 349
Comvie, 84
Concepcion, 150, 151, 158, 235, 269, 299, 327, 332, 337
Conklin, 270, 288
Cook, 84
Cooper, 13, 31, 53, 56, 82, 115, 116, 117, 121, 128, 130, 131, 132, 138, 152, 153, 155, 160, 164, 180, 205, 211, 269, 330, 331, 332, 333, 335, 337, 343
Coplans, 224
Corson-White, 181
Coutts, 286, 287
Couzien, 204, 210
Coward, 24, 57, 58, 66, 67, 70, 71, 74, 76, 78, 90, 94, 322, 324, 329
Cowgill, 177
Cox, 205
Cramer, 71, 180, 183
Crawford, 32
Crowell, 205, 337
Curatolo, 333
Curran, 86, 270, 301, 345, 348
Czerny, 235

Dahle, 86, 276, 277
Dalyell, 192, 232, 306, 309
Damon, 160
Daniels, 56, 69, 98, 120, 121, 157, 158, 278, 281, 282, 283, 287, 296, 322, 323, 326, 329, 335, 342, 343
Darling, 220
Dass, 148
Davey, 103, 108, 232
Davidsohn, 193, 310
Davis, 3, 12, 13, 15, 16, 23, 26, 28, 30, 32, 35, 46, 47, 48, 56, 57, 61, 63, 64, 83, 84, 85, 86, 89, 90, 95, 96, 97, 111, 112, 113, 114, 115, 116, 150, 151, 154, 157, 158, 162, 164, 168, 210, 213, 269, 280, 290, 317, 320, 321, 322, 323, 324, 326, 327, 332, 333, 335, 337, 338, 340, 341, 342, 346, 347, 348, 349, 350, 351, 353
Dawson, 245
Dean, 278
Degrez, 180
De Korte, 173
De Lacerda, 207
De Langen, 202
Delf, 84, 92, 99, 100, 101, 103, 106, 133, 134, 185, 305, 306, 327, 334, 347, 349
DeMello, 205, 206
Denis, 131, 261
Dennis, 262
Denton, 101, 157
Devloo, 43, 313
Dick, 146, 192
Diesing, 195
Di Mattei, 343
Doan, 278
Donaldson, 19, 22
Douglas, 171, 173, 174, 185, 211
Drew, 183
Drummond, 4, 16, 24, 25, 31, 57, 58, 66, 67, 69, 70, 71, 74, 76, 78, 82, 90, 91, 94, 112, 113, 115, 117, 118, 120, 124, 127, 128, 130, 149, 153, 157, 158, 166, 167, 169, 173, 174, 175, 179, 180, 182, 194, 221, 237, 293, 295, 296, 297, 320, 321, 322, 323, 324, 329, 330, 331, 332, 333, 337, 346
Dubin, 17, 31, 40, 147, 151, 159, 160,

179, 212, 238, 257, 314, 316, 319, 333, 336, 338, 343
Ducaisne, 272
Dudgeon, 195
Dutcher, 67, 86, 88, 89, 103, 104, 128, 133, 171, 174, 182, 187, 271, 276, 277, 279, 284, 343, 344, 345, 348
Dyke, 84, 353

Eckles, 67, 86, 103, 272, 273, 276, 277, 279, 284
Eddy, 17, 39, 42, 82, 116, 118, 131, 139, 141, 160, 297, 314, 331, 333
Edie, 122, 130, 179, 337, 339, 343
Edkins, 186
Eijkman, 12, 13, 18, 94, 97, 115, 117, 153, 180, 203, 204, 337
Ellis, 100, 113, 277, 291
Emmett, 36, 43, 44, 95, 96, 97, 98, 118, 154, 158, 159, 160, 163, 169, 171, 173, 185, 207, 237, 238, 239, 267, 281, 314, 323
Engel, 192, 273
Engstrand, 100, 221, 223, 349
Enright, 259
Esser, 195
Euler, 314
Eustis, 130
Evans, 122, 130, 337, 343
Ewing, 25, 56
Eykman, 155, 156

Faber, 353
Fahrion, 113, 335
Falk, 107
Falta, 7, 8, 234
Fargier, 205
Ferguson, 195
Ferry, 55, 115, 120, 130, 157, 162, 333, 336
Fildes, 317
Findlay, 187, 190, 191, 192, 193, 195, 196, 211, 228
Fine, 246
Fingerling, 274
Fink, 329
Finkelstein, 279
Finks, 49, 56, 73, 74, 77, 327, 335, 338, 342
Fish, 298, 350
Fishback, 157
Fitch, 164, 216
Fleischmann, 37
Fleming, 315
Fletcher, 204, 214
Folin, 114, 131, 261
Forte, 193
Fox, 88
Fraenkel, 222
Fraga, 201, 203, 204
Francis, 248
Frank, 237, 240
Frankel, 43, 142, 228
Fraser, 13, 79, 115, 116, 119, 131, 153, 204, 337
Freedman, 160
Freise, 240
Frölich, 100, 103, 104, 105, 107, 132, 134, 135, 201, 217, 220, 221, 222, 223, 228, 230, 284, 347, 350, 351
Fulmer, 43, 314
Funk, 3, 4, 13, 14, 15, 16, 17, 25, 28, 29, 31, 40, 57, 77, 97, 98, 114, 115, 116, 118, 121, 122, 123, 124, 126, 127, 128, 130, 131, 139, 151, 152, 153, 159, 160, 167, 171, 173, 174, 179, 180, 182, 185, 205, 211, 212, 213, 214, 221, 223, 238, 256, 257, 280, 314, 316, 319, 333, 335, 336, 337, 338, 342, 343, 344, 345, 350, 352
Fürst, 83, 105, 223, 226, 349

Gaglio, 333
Gams, 140
Ganassini, 162
Garrison, 248, 260
Gerstenberger, 222, 227, 228, 353
Gibson, 150, 151, 158, 173, 207, 269, 299, 332, 337
Giorgi, 286
Givens, 85, 100, 101, 102, 106, 107, 108, 109, 134, 135, 144, 147, 222, 224, 245, 246, 344, 347, 350, 351, 352
Glisson, 190
Goldberger, 242, 243, 248, 249, 252, 253, 254, 256, 260
Golding, 70, 194
Goldschmidt, 237, 240
Goto, 210
Goy, 316
Green, 31, 32, 56, 79, 80, 162, 326, 335, 336

Grieg, 134, 204, 215, 343
Grijns, 94, 97, 124, 155, 156
Grim, 248
Gross, 20, 31, 56, 63, 66, 72, 73, 75, 81, 165, 181, 182, 207, 237, 324, 325, 328, 334, 335, 340, 341
Guerrero, 235, 327

Haguenea, 218, 223
Hale, 323
Hall, 104, 133
Halliburton, 67, 113, 237, 320, 321, 322, 323, 324
Halpin, 21, 56, 207
Hammersten's, 23
Hampshire, 296
Hansemann, 195
Hansen, 6, 21, 23
Harden, 40, 104, 108, 118, 128, 129, 132, 143, 144, 151, 166, 170, 221, 276, 298, 342, 352, 353
Harley, 87, 223
Harrie, 246, 256
Harshaw, 104, 133
Hart, 15, 21, 26, 32, 47, 48, 56, 86, 100, 104, 207, 265, 272, 277, 284, 286, 290, 291, 327, 346, 348, 353
Hartwell, 294
Haussler, 187
Hawk, 148, 149, 157, 181, 263, 305, 311, 329, 343, 353
Hawker, 296
Hawkins, 217
Heft, 42, 314
Hefti, 137
Hehir, 87
Henriques, 6, 21, 23
Henry, 313
Hess, 83, 86, 101, 102, 104, 109, 110, 132, 133, 134, 169, 192, 194, 195, 198, 218, 219, 220, 222, 226, 236, 237, 260, 274, 276, 277, 284, 285, 286, 290, 293, 298, 309, 310, 345, 346, 348, 349, 350, 351, 352, 353
Hewlett, 173
Highet, 214
Hindhede, 53, 264
Hirota, 202
Hitchcock, 131
Hittcher, 268, 283
Hoffman, 224
Hofmeister's, 7, 125
Hogan, 56, 63, 281
Holm, 182
Holst, 95, 97, 100, 103, 105, 107, 132, 134, 135, 155, 156, 201, 217, 220, 221, 222, 223, 228, 230, 338, 339, 343, 344, 345, 347, 350, 351
Holt, 194, 279
Hopkins, 3, 6, 10, 11, 14, 23, 24, 29, 34, 56, 70, 91, 94, 151, 175, 185, 186, 209, 237, 238, 269, 276, 288, 299, 309, 332, 344, 346
Horbaczevski, 247
Hort, 279
Howard, 194, 229
Howe, 178
Howland, 199
Huessy, 311
Hughes, 88, 164
Hulshoff, 215
Hume, 18, 25, 34, 78, 82, 83, 85, 86, 88, 89, 95, 96, 98, 99, 104, 150, 151, 152, 155, 156, 164, 165, 221, 254, 257, 259, 269, 270, 277, 283, 284, 286, 290, 298, 307, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353
Humphrey, 26, 32, 48, 265
Hunter, 138, 245, 246, 256
Huynen, 193

Ide, 313
Igaravidez, 205, 206
Ingier, 220
Ingvaldsen, 229
Izume, 210

Jackson, 87, 222, 223, 224, 345
Jacob, 7
Jansen, 143, 216, 323
Jelliffe, 179, 201, 206
Jephcott, 271, 291
Jobling, 248
Johansen, 87
Johns, 49, 56, 73, 74, 77, 327, 329, 335, 338, 342
Johnson, 42, 314
Johnston, 209, 210
Jones, 254
Just-Hatmaker, 104, 286, 290, 291

Kaoliang, 124
Karr, 96, 176, 177, 180, 184, 207, 208, 226
Kassowitz, 195
Kastle, 56
Keeton, 186
Keller, 235
Kempster, 76
Kennedy, 16, 62, 63, 67, 76, 77, 115, 117, 151, 153, 162, 165, 276, 332, 334, 336, 340
Kent, 20, 34, 56, 63, 67, 70, 91, 165, 207, 237, 326, 335
Kilbourne, 205
Kimura, 124
Klose, 193
Knapp, 234
Knowles, 311
Koch, 186, 193
Koga, 345, 348, 351, 352
Kohman, 101, 157, 170
Kohn, 274
Koning, 273, 274

Labbe, 218, 223
La Cellule, 43
Laeger, 53
Lake, 18, 78, 79, 119, 150, 156, 158, 207, 208, 330, 335, 336, 338, 339, 344
Lamb, 234, 238
Lamer, 33, 100, 102, 111, 134, 228
Lane-Claypon, 262, 263, 264, 268, 270, 272, 273, 274, 281, 287, 288, 299
Langstein, 191
Lapicque, 208
Lassabliere, 295
Lavinder, 248
Leavenworth, 120
Lebredo, 210
Legroux, 318
Levy, 41
Lewis, 83, 226, 245, 246, 351
Liebig, 313
Lienaux, 193
Lind, 83, 86, 229
Linossier, 316
Little, 201, 205, 214
Lloyd's, 40, 118, 139, 317
Loeb, 82, 341
Loew, 59
Lorenz, 248
Loughlin, 56, 69, 278, 281, 283, 287, 296, 322, 323, 329, 342, 343
Loundro, 205, 206
Luckett, 14, 18, 29, 63
Lumiere, 164, 184, 315
Lunin, 2, 6
Luros, 95, 96, 97, 98, 154, 158, 267, 281, 323
Lux, 186
Lyle, 31, 212

Macallum, 14, 25, 28, 57, 97, 114, 130, 131, 152, 153, 211, 212, 238, 280, 344
MacDonald, 43, 314
Mackay, 163, 194, 196, 199
Macomber, 174
Macy, 109, 144, 147, 351, 352
Madsen, 169, 174
Mancini, 162
Mangkoewinoto, 216
Marfan, 193
Marsh, 261
Mathews, 186, 262
Matti, 193
Mattill, 174, 270, 288
Mayer, 170, 313
McArthur, 14, 18, 29, 63
McCann, 169, 237
McCarrison, 164, 169, 170, 171, 172, 173, 174, 177, 178, 179, 181, 185, 188, 205, 207, 209, 292, 309, 310, 311
McCaskey, 31, 212
McClendon, 100, 133, 137, 144, 146, 147, 149, 220, 223, 349, 352
McClugage, 85, 101, 102, 107, 108, 109, 134, 135, 344, 350, 351, 352
McClurg, 98, 120, 121, 157, 158
McCollum, 3, 7, 8, 12, 13, 15, 16, 19, 20, 21, 22, 23, 26, 28, 31, 32, 41, 42, 43, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 61, 63, 64, 65, 66, 73, 74, 77, 78, 82, 90, 93, 95, 97, 98, 111, 112, 113, 114, 115, 116, 117, 118, 119, 129, 150, 151, 153, 154, 157, 158, 160, 162, 163, 164, 165, 166, 168, 177, 181, 183, 186, 195, 199, 207, 210, 213, 220, 222, 225, 226, 234, 239, 240, 241, 257, 259, 264, 266, 269, 272, 275, 276, 280, 293, 294, 303, 304, 307, 314, 320, 321, 322, 323, 324, 325, 326, 327, 328, 332, 333, 334, 335, 336, 337, 338, 340, 341

McGuire, 107
McKim, 118, 154
McLeod, 318
McNab, 353
McNeal, 247, 248, 253, 260
Mead, 86, 276, 277
Medes, 109
Meigs, 261
Mellanby, 168, 177, 178, 194, 195, 196, 197, 198, 292
Mendel, 3, 7, 8, 10, 11, 12, 13, 15, 17, 21, 22, 23, 24, 25, 27, 30, 31, 32, 34, 38, 48, 49, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 71, 72, 73, 77, 78, 80, 81, 82, 90, 91, 92, 93, 97, 111, 112, 114, 115, 119, 136, 137, 150, 151, 153, 157, 158, 162, 164, 166, 167, 168, 171, 175, 176, 179, 186, 194, 221, 226, 236, 237, 238, 239, 240, 256, 266, 268, 269, 270, 276, 297, 320, 321, 322, 323, 324, 325, 326, 328, 329, 330, 331, 332, 333, 334, 335, 336, 338, 339, 340, 341, 342, 344, 345, 346, 347, 348, 349, 352, 353
Menzies, 170
Merrell-Soule, 147, 287, 290
Mewer, 191
Meyers, 246
Michel, 97, 155
Middlekauf, 100, 221, 223, 349
Miles, 174
Miller, 32, 48, 95, 96, 120, 160, 199
Millon, 60
Minot, 261, 262
Mircoli, 193
Mitchell, 23, 25, 56, 60, 61, 62, 63, 152, 153
Miura, 68, 134
Monrad, 235
Moody, 224
Moore, 122, 130, 222, 337, 343, 345
Morgan, 56
Morikawa, 219
Morner, 280
Mori, 235
Mortram, 183
Moskowski, 210, 211
Moszkowski, 15
Mouriquand, 84, 95, 97, 155, 205, 208, 348, 349
Moussu, 193
Muckenfuss, 333
Munk, 60
Munson, 350, 352
Murlin, 246
Myers, 49, 78, 79, 80, 96, 116, 117, 118, 126, 130, 132, 150, 186, 256, 313, 327, 330, 335, 336, 338, 339, 344

Nageli, 35, 39, 40, 313
Naish, 288
Nansen, 87
Neill, 138, 256
Nelson, 23, 43, 60, 61, 62, 63, 75, 94, 234, 238, 314
Nepveux, 218, 223
Neumann, 104
Neusser, 247
Neville, 11, 151, 269, 332
Nichols, 56, 326, 335
Nicolaidi, 245
Nobel, 219
Noeggerath, 7, 8, 234
Nollau, 56
Norman, 299
Northrop, 82, 341
Novaro, 162, 176

Odake, 123, 130, 131, 337
Ohler, 205, 339
Osborne, 3, 7, 8, 10, 11, 12, 13, 15, 17, 21, 22, 23, 24, 25, 27, 30, 31, 32, 34, 38, 48, 49, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 71, 72, 73, 77, 78, 80, 81, 82, 90, 91, 92, 93, 97, 111, 112, 114, 115, 119, 120, 130, 136, 137, 150, 151, 153, 157, 162, 164, 166, 167, 168, 171, 175, 176, 179, 194, 236, 237, 238, 239, 240, 256, 262, 264, 266, 268, 269, 270, 276, 297, 320, 321, 322, 323, 324, 325, 326, 328, 329, 330, 331, 332, 333, 334, 335, 336, 338, 339, 340, 341, 342, 344
Oshima, 203
Ottow, 212
Outhouse, 168

Pacini, 160, 317
Padua, 179
Page, 148
Palmer, 76, 77, 115, 272, 273
Pappenheimer, 64, 169, 237
Park, 65, 177, 195, 199, 279
Parker, 206

Parsons, 26, 28, 31, 49, 51, 52, 55, 73, 78, 95, 151, 164, 166, 177, 195, 199, 220, 221, 239, 257, 266, 320, 323, 327, 328, 337, 341, 345
Pasteur, 35, 313
Paton, 113, 195, 199
Paul, 329, 342
Pavlov, 7
Payne, 88
Paynton, 194
Peronne, 84
Peronnet, 349
Peterson, 248
Pettersson, 314
Phemister, 199
Phillips, 56
Pierson, 86, 88, 344, 345, 348
Pilgrim, 288
Pitz, 20, 31, 32, 48, 49, 52, 74, 77, 82, 98, 111, 112, 158, 160, 222, 225, 226, 228, 272, 276, 323, 324, 326, 327, 334, 335, 336, 338, 344
Plauson, 143
Plaut, 273
Plimmer, 56, 221
Pol, 124, 215
Pollini, 246
Pollock, 245
Polyclinico, 333
Portier, 181
Potter, 168
Preti, 246
Price, 278
Pritchard, 293, 296
Pugliese, 228

Quincy, John 1

Rae, 87
Ramoino, 187
Raubitschek, 247
Ray, 32, 109
Read, 74, 92
Rebello, 205, 206
Rehfuss, 311
Renshaw, 305
Renton, 193
Rettger, 280
Reynolds, 174
Rhodes, 83, 85, 102, 288, 298, 347, 349, 350, 351, 352, 353
Rich, 56
Richardson, 32, 56, 114, 326, 335
Riddell, 205, 206
Ridlon, 254, 255
Rimbaud, 201, 207, 209
Rivers, 317
Robb, 229
Roberts, 242, 245, 247, 252, 255
Robertson, 193
Robinson, 144
Robison, 104, 108
Roger, 201, 207, 209
Rohmann, 8, 16, 17, 18, 56, 59
Rommel, 207
Rondoni, 247, 256
Ronne, 235
Roof, 245, 259
Roper, 118, 141, 297
Rose, 53, 56
Rosenheim, 76
Rosenstein, 76
Rossi, 103
Rouse, 288
Rubner, 59
Ruhl, 247
Rumsey, 274
Russell, 160, 317

Saikingakuzashi, 132
Saleeby, 118, 216
Salvatore, 193
Sambon, 247
Sanctorius, 1
Sawamura, 213
Saxon, 181
Scala, 59
Schaefer, 86, 276, 277
Schaeffer, 292
Schaumann, 97, 121, 151, 156, 211
Schicht, 138
Schmidt, 192
Schmorl, 192
Schreiber, 140
Schut, 202
Schwarz, 43, 142
Schweiz, 187
Schweizer, 44, 312
Scola, 247
Scott, 87, 130
Seaman, 331
Sedgwick, 144, 352

Seidell, 40, 77, 98, 118, 119, 127, 128, 131, 141, 153, 154, 160, 182, 216, 344
Sekine, 283, 321, 322
Sell, 67, 69, 75, 76, 92, 94, 113
Sharp, 133
Shaw, 148
Shearer, 317
Sherman, 33, 56, 64, 100, 102, 134, 204, 261, 264, 265, 288
Sherwood, 43, 314
Shibayama, 210
Shimamura, 123, 130, 131, 132, 337
Shipley, 65, 177, 195, 199
Shorten, 109
Sicard, 201, 207, 209
Siegert, 193
Siler, 243, 248, 254, 260
Simmonds, 20, 26, 28, 31, 32, 48, 49, 50, 51, 52, 55, 61, 63, 65, 73, 74, 77, 78, 82, 95, 98, 111, 112, 116, 117, 118, 119, 129, 151, 154, 157, 158, 160, 163, 168, 177, 183, 195, 199, 234, 239, 240, 257, 259, 266, 272, 275, 276, 293, 320, 323, 324, 325, 326, 327, 328, 334, 335, 336, 337, 338, 341
Simonnet, 216
Simpson, 122, 130, 179, 201, 337, 339, 343
Singer, 245
Skelton, 18, 83, 85, 88, 92, 106, 284, 298, 345, 347, 349, 350, 351, 352, 353
Slonaker, 19
Smith, 84, 86, 104, 109, 148, 181, 205, 206, 219, 284, 286, 290, 305, 329, 343, 346, 348, 353
Socin, 8
Soldner, 283
Solomin, 283
Sousa, 41, 42
Souza, 314
Soxhlet, 268
Sprawson, 209
Stammers, 74, 92
Stanton, 13, 79, 115, 116, 119, 131, 153, 204, 245, 337
Starling, 185
Steenbock, 20, 21, 26, 31, 32, 34, 48, 56, 63, 66, 67, 69, 70, 72, 73, 75, 76, 81, 86, 91, 92, 94, 100, 104, 111, 112, 113, 117, 118, 119, 128, 131, 136, 155, 165, 167, 181, 182, 207, 226, 234, 237, 238, 265, 277, 284, 286, 290, 291, 307, 324, 325, 326, 328, 332, 334, 335, 340, 341 346, 348, 353.
Stefanson, 87, 99, 218
Stephenson, 67, 238
Stepp, 8, 14, 97, 115, 116, 272
Stevenson, 39, 42, 234, 238, 314, 333, 345, 346, 348
Still, 298
Stockholm, 36, 43, 159, 160, 314
Stoeltzner, 193, 194
Strong, 205, 337
Stuessy, 282
Sturtevant, 237
Suarez, 247
Suguira, 82, 98, 115, 117, 125, 131, 151, 161, 208, 309, 329, 340, 341, 351
Sullivan, 79, 80, 118, 124, 132, 245, 254, 256, 330, 337, 339
Sundwall, 245
Supplee, 274, 277
Sure, 50, 74, 92, 267
Suzuki, 123, 130, 131, 132, 337
Swalingam, 245
Sweet, 181
Swoboda, 37, 82, 182, 185, 314, 330, 331
Sydenstricker, 249, 256

Takahata Chuo, 210
Takaki, 202, 203, 214
Talbot, 223
Telfer, 177
Terunchi, 132
Tervvchi, 115
Theiler, 31
Theze, 214
Thjotta, 160, 317
Thomas, 51, 53, 258, 264
Thompson, 245, 249, 256, 260
Tobler, 220
Torane, 193
Totani, 56, 238
Towels, 14, 152, 182
Tozer, 99, 163, 194, 197
Tschirch, 187

Ugesk, 53
Uhlmann, 182, 184
Underhill, 253, 256, 257
Unger, 83, 86, 101, 104, 109, 132, 133, 134, 194, 195, 198, 222, 226, 260, 274, 276, 277, 290, 345, 346, 348, 351, 352

Van Alstyne, 181
Van der Wielen, 343
Van Leent, 214
Van Leeuwen, 216
Van Slyke, 263
Veeder, 115, 116, 117, 118, 124, 127, 130, 131, 132, 150, 151, 155, 180, 183, 205, 207, 213, 214, 245, 248, 249, 250, 252, 256, 336, 337, 340, 343
Verzar, 216
Vezar, 173, 183
Vielle, 143
Viljoen, 31
Vinson, 201
Virchow, 191
Viswalingam, 259
Voegtlin, 14, 18, 49, 78, 79, 80, 96, 116, 117, 118, 119, 124, 126, 128, 130, 132, 138, 150, 152, 156, 158, 182, 186, 207, 208, 246, 247, 256, 313, 327, 330, 335, 336, 337, 338, 339, 344
Vogt, 193
Volpio, 247
Von Groer, 163, 194, 236
Von Schonborn, 211
Vorderman, 214

Wakeman, 114, 115, 119, 120, 130, 157, 262, 264, 333, 336
Walshe, 211
Waring, 252
Wason, 234
Watson, 74, 195, 199
Webster, 122, 130, 337, 343
Weill, 84, 95, 97, 155, 205, 208, 348, 349
Weiser, 214
Wellman, 205, 339
Wells, 25, 56, 223
Wheeler, 30, 56, 60, 249, 253, 256
Whipple, 36, 96, 118, 120, 314
White, 128
Wilbur, 103, 279, 284
Wildiers, 43, 313
Wilkins, 174
Willaman, 76, 315
Willcock, 6
Willcox, 86, 344, 345, 352, 353
Willets, 252
Williams, 35, 37, 38, 39, 41, 95, 96, 116, 118, 119, 120, 121, 124, 127, 128, 129, 130, 131, 150, 151, 154, 158, 159, 182, 205, 209, 210, 213, 314, 331, 332, 333, 334, 339, 340, 341, 342, 344
Wilson, 258
Wiltshire, 84, 86, 88, 110, 344, 348
Winfield, 286, 288
Winters, 56
Withers, 32
Woll, 273
Wood, 245, 256, 288
Wright, 223
Wydooghe, 201, 209
Wyon, 318

Yarborough, 247
Yates, 56

Zilva, 40, 68, 70, 91, 118, 128, 129, 132, 134, 136, 143, 151, 166, 170, 221, 223, 298, 324, 328, 340, 342, 350, 352, 353
Zuckmayer, 274
Zunz, 309

INDEX OF SUBJECTS

Abdominal walls, effect of lack of vitamins on, 173
Absorption, selective, separation of *B* and *C* by, 132
Absorption of *B*, 117, 118
Accessory food substances, 3
Acetic acid extract of yeast, 119
Acetic acid solution of *B*, fractionation by alcohol, 115, 116
Acetone, solubility of *A* in, 111
Acetone, solubility of *B* in, 116, 117, 153
Acid character of *B*, 130
Acid, extraction of *A* from oils by, 112
Acid hydrolysis, effect on rice polishings, 118, 119
Acid, stability of *B* towards, 118, 154
Acid, thermostability of *C* in presence of, 102 (footnote), 134, 135
Acidity of diet, effect on growth, 48
Acidosis a factor in rickets, 193, 194
Acidosis a result of lack of *B*, 172
Acidosis, relation of vitamin deficiency to, 310
Acne rosacea, effect of yeast on, 311
Acne vulgaris, effect of yeast on, 311
Activated fuller's earth, 141, 154
Activated fuller's earth, growth-promoting and antineuritic power compared, 154
Adenine, antineuritic properties of, 127, 182
Adrenaline, effect of lack of *B* on production of, 171
Adrenaline, effect of lack of *C* on production of, 177
Adrenalin, production in polyneuritis, 207
Adrenaline, relation to oedema, 170
Adrenals, effect of lack of vitamins on, 171, 172, 173, 177, 181, 228 (footnote)
Adrenals, enlargement of in polyneuritic, 170, 207
Advitants, 4
Aeration, effect on *A* in butter-fat, 90, 91
Aeration, effect on *A* in oils, 90
Aeration of milk during pasteurization, effect on nutritive properties, 285
Aeration, stability of *C* toward, 100, 103, 104
Aerobic and anaerobic yeasts, vitamin requirements of, 36
Agar an ingredient of experimental diets, 8, 14, 22
Age, variation of nutritive value of vegetables with, 101
Age, variation of vitamin requirements with, 175
Aging of milk, deleterious effect of, 284, 295
Albumin, bacterial growth-stimulant in, 160
Albumin in heated milk, precipitation of, 281
Albumoses, 126
Alcohol as solvent for *A* in plant tissues, 136
Alcohol, fractionation of acetic acid solution of *B* by, 115, 116
Alcohol, methyl, solubility of *B* in, 116
Alcohol, solubility of *B* in, 115, 116, 117, 153
Aleurone layer of cereal grains, *B* in, 78
Alfalfa as source of *A* ,72, 73, 136
Alfalfa, dried, as source of *A*, 137
Alfalfa, content of *B* in, 81
Alfalfa, extraction of *A* from by ether, 111, 112
Alfalfa flour, 52
Alfalfa, thermostability of *A* in, 92
Algae, synthesis of *A* by, 77
Alkali, stability of *A* toward, 90
Alkali, stability of *B* toward, 96, 119, 120, 154
Alkali, stability of *C* toward, 133, 134
Alkali, use in isolation of *B*, 120, 124
Alimentary products rich in *B*, preparation of, 142
Allantoin, 122
Allantoine, antineuritic properties of, 127, 182
Almond, content of *A* in, 74
Aluminum silicate (Lloyd's reagent) as absorbent of vitamin *B*, 118
Amanita muscaria, relation of vitamins to growth of, 315

Amenorrhea, 174
Amino-acids, xi, 2, 54
Amino acids a limiting factor in growth, 54
Amino acids, importance in nutrition, 267, 268
Amino acids in the leaf, 52
Amino acids in tubers, 54
Amino acids of barley proteins, 55
Amino acids of maize protein, 55
Amino acids of milk proteins, 266
Amino acids of oat protein, 55
Amino acids of rice proteins, 55
Amino acids, relative importance of, 257, 258, 259
Amino acids, stimulating effect on yeast growth, 42
Anaemia, deficiency of vitamins a factor in, 168, 169, 173, 174
Anaerobic yeasts, vitamin requirements of, 36, 187
Animal as compared with vegetable protein, biological value of, 258
Animal fats, antirachitic value of, 197, 198
Animal fats, stability towards alkali of *A* in, 113
Animal foods, antiscorbutic value of, 232
Animal husbandry, food value of various proteins in, 265
Animal products, antiscorbutic value of, 86
Animal products, content of *B* in, 82
Animal tissues, dried, content of *A* in, 91, 92
Animals susceptible to beriberi, 207, 208
Antineuritic and growth-promoting factors, comparison of thermostability of, 154 and ff
Antineuritic as distinct from growth-promoting factor, 150 and ff
Antineuritic factor as nerve stimulant, 182, 183
Antineuritic foods, 215
Antineuritic properties of adenine, 127
Antineuritic properties of allantoin, 127
Antineuritic properties of betaine, 127
Antineuritic properties of choline-like bases from rice, 127
Antineuritic properties of cinchonine, 128
Antineuritic properties of hydroxy-nicotinic acid, 128
Antineuritic properties of isomeric hydroxy-pyridines, 128, 129
Antineuritic properties of nicotinic acid, 127
Antineuritic properties of pyrimidines, 127
Antineuritic properties of pyrimidine derivatives, 127, 128
Antineuritic properties of purine-like bases from rice, 127
Antineuritic properties of quinine, 128
Antineuritic properties of tethelin, 128
Antineuritic properties of trihydroxy pyridines, 128
Antineuritic requirement, effect of carbohydrate on, 179, 180
Antineuritic substances, preparation of crystalline, 121 and ff
Antineuritic value of certain foodstuffs, table of comparative, 82
Antineuritic vitamins, two, 213
Antirachitic factor, 168, 170, 194, (footnote), 196 and ff
Antiscorbutic foods for infants, 298
Antiscorbutic requirements of different species, 166
Antiscorbutic value as affected by preservation of foods, 99
Antiscorbutic value of foodstuff enhanced by presence of *A*, 99, 100
Antiscorbutic value of milk, 277
Antiscorbutic vitamin, quantitative determination of, 33
Apples, content of vitamin *B* in, 82, 171 (footnote)
Appetite a factor in nutrition experiments, 7
Appetite and growth, relation between, 175, 176
Appetite, effect of *A* on, 167
Appetite, effect of *B* on, 175, 176
Appetite-promoting factor, 177
Appetite, relation of growth to, 10
Appetite, relation of vitamins to, 309
Argenine, 259
Arthritis deformans, effect of yeast on, 311
Aspergillus niger, relation of vitamins to growth of, 315
Assimilation, effect of lack of *B* on, 171, 172
Atoxyl, 242 (footnote)
Asthenia, lack of vitamins, a cause of, 173
Atrophic disorders, lack of vitamins, a cause of, 309
Atrophy of myenteron, relation of vitamin deficiency to, 311
Atropine, 183
Atropine, inhibition of glandular secretion by, 184

Auerbach's plexus, relation of vitamin deficiency to degeneration of, 311
Autoclave, temperature of, 89
Autoclaved milk as constituent of scorbutic diet, 104
Autolyzed yeast, antineuritic value of, 77
Avitaminosis in children, 292 and ff

Bacillus coli, 160
Bacillus de Pfeiffer, 317, 318
Bacillus influenza, 160
Bacillus paratyphosus *B*, 160
Bacillus subtilis, 160
Bacillus typhosus, 160
Bacteria vitamin requirements of, 317, 318
Bacteria, synthesis of *B* by, 160, 161
Bacterial growth, relation of vitamins to, 160, 161
Bacterial growth-stimulant, 160, 161
Bacterial infection in scurvy, 224, 225
Bacterial infection in pellagra, 247, 248
Balanced diet, necessity for, 240
Balancing of rations, 32
Banana, content of *A* in, 74
Banana, content of *B* in, 82
Banana, content of *C* in, 83
Banana flour, preparation of, 107
Barley, antineuritic and growth-promoting power compared, 152
Barley, antineuritic value of, 215
Barley, a constituent of antiscorbutic ration, 83
Barley protein, amino acids of, 55
Barley protein in the diet, 51
Barley, thermostability of *B* in, 95
Barlow's disease, 219, 220
Basal diets deficient in *A*, 168
Basal diets, faulty character in early work, 46
Basal diets, 44, 57 and ff
Basal diets, preparation of, 44
Basal diets, purification of ingredients in, 57
Basal diets, salt mixtures used in, 59 and ff
Basal metabolism, xiii
Base content of diet, limits of, 48
Basic character of *B*, 130
Beans, alcohol extraction of *B* from, 116, 117
Beans as antineuritic, 205
Beans as antiscorbutics, 232
Beans, biological value of protein of, 258
Beans, content of *B* in, 77, 205
Beans, cooked, deficiency in *B*, 116
Beans, cooked, retention of *B* by, 116
Beans, dried, antiscorbutic value of, 83
Beans, Georgia velvet, content of *A* in, 73, 74 (footnote)
Bean, Georgia velvet, content of B in, 74 (footnote)
Bean, Georgia velvet, thermostability of *A* in, 92
Bean, injurious effect on rat, 49
Bean Katjangidjo, thermostability of *B* in, 94
Beans, effect of alkali on *B* in, 120
Beans, navy, effect of cooking with sodium bicarbonate on *B* in, 120
Beans, navy, thermostability of *B* in, 95, 96, 98
Bean protein, biological value of, 49, 51
Beans, soy, thermostability of *B* in, 98
Beans, string, content of *C* in, 85
Beans, string, canned, content of *C* in, 85
Beans, string, thermostability of *C* in, 100
Beans, string, variation in antiscorbutic value of, 84
Beef, content of vitamin *B* in, 119
Beef, dried, antiscorbutic value of, 109
Beef fat, content of *A* in, 67, 71
Beef, heated, beriberi produced by diet of, 208
Beef infusion, bacterial growth-stimulant in, 160
Beef muscle, antineuritic value of, 82
Beef, thermostability of *B* in, 97
Beer, antiscorbutic value of, 84
Beet sugar, content of *A* in, 73, 75
Beet sugar, content of *B* in, 82
Beets, general nutritive character of, 53
Beets, content of *B* in, 81
Beets, content of *C* in, 85
Benzene as solvent for *A* in plant tissues, 136
Benzene, solubility of *A* in, 111, 112
Benzene, solubility of *B* in, 117, 153
Beri-beri, 3, 12, 13, 201 and ff
Beriberi, abnormal phosporic acid content of urine in, 211
Beriberi among Philippine scouts, 205
Beriberi and scurvy, clinical link between, 218, 219, 220
Beriberi, animals susceptible to, 207, 208
Beriberi, areas of infection in Africa, 209
Beriberi, autolyzed yeast as preventive of, 77
Beriberi, chronic and acute, 208, 209
Beriberi, dietary hypothesis, 214

Beriberi, diets producing, 203 and ff
Beriberi "dry", 206, 213
Beriberi, etiology of, 203, 204, 209, 210
Beriberi identical with polyneuritis, 203, 204
Beriberi in breast-fed infants, 202
Beriberi, incidence of, 201, 202
Beriberi, incidence among women, 202
Beriberi, infection theory of, 202, 209, 210
Beriberi-like symptoms in calves, 165
Beriberi, mineral metabolism in, 59
Beriberi, outbreak at Elizabeth, N.J., 206
Beriberi, outbreak at Kutel-Amara, 206
Beriberi, outbreaks in Labrador and Newfoundland, 206
Beriberi, predisposition to, 201, 202, 204
Beriberi produced by diet of wheat endosperm, 78
Beriberi, study in Japanese navy of, 202, 203
Beriberi, symptoms of, 206, 207
Beriberi, theories of origin, 203, 204, 209, 210, 211, 212
Beriberi toxin, 210, 211
Beriberi, wet, 206, 207, 213
Betaine, 125
Betaine, antineuritic properties of, 127, 182
Bezssonoff test for *C*, 135
Biliary insufficiency, relation of vitamins to, 172
Bile, stimulation of flow of by *B*, 186
Bilious vomiting, relation of vitamin deficiency to, 310
Biological analysis of foods, 46
Biological value of cereal and legume mixtures, 50
Biological value of proteins, 257, 258, 259
Biologic values of proteins, 258, 264, 265, 266, 267
Bios, 43, 313
Bios, relation of *B* to, 313
Biosine, 313 (footnote)
Bladder, effect of lack of *C* on, 177
Blindness in calves from lack of vitamin, 165
Blood count of rats deprived of *A*, 168
Blood, effect of lack of *B* on nitrogen of, 174
Blood, effect of vitamin deficiency in red cells of, 168, 174
Blood, effect of lack of *B* on total solids in, 174
Blood, effect of lack of *B* on urea in, 174
Blood, predisposition to beriberi due to excess sugar in, 202
Blood pressure, effect of vitamin extracts on, 183
Blood vessels, effect of vitamin extracts on, 183
Boiled milk, digestibility of, 279
Boiled milk, nutritive value of, 279, 282, 283
Bolted flour a cause of beriberi, 206
Bones, effect of lack of *A* on, 169, 170, 177
Bones, effect of lack of *B* on, 178
Bones, effect of lack of *C* on, 178, 221, 222
Bones, effect of vitamins on calcification of, 177
Bones, rachitic-like derangements of, 199
Bowel, effect of lack of *B* on, 172
Brain, content of A in, 91, 92
Brain, content of *B* in, 82, 117, 152
Brain, effect of lack of *B* on, 171, 173
Brain, growth-promoting and antineuritic power compared, 152
Bran, content of *A* in, 74
Bran, content of *B* in, 74, 78
Bran, rice, content of *B* in, 78
Bran, wheat, content of *B* in, 78, 82
Brazil nut, content of *A* in, 74
Breakfast foods, vitamin deficiency of, 80
Bread, nutritive properties of soda biscuit compared with, 120
Bread, white, in beriberi producing diets, 205
Breast-fed children, high mortality of artificially-fed as compared with, 299
Breast-feeding, relation of rickets to, 193
Breast milk of pellagrins, 246
Breast milk, vitamin deficiency in, 293, 294
Breeding stock, necessity of selection of, 28, 29
Brewers yeast, 14
Bronchitis, effect of yeast on, 311
Butter a protection against oedema, 170
Butter as source of *A*, 66, 67, 74, 91, 301
Butter fat an ingredient of experimental diets, 3, 8, 12, 14, 15
Butter fat, antineuritic value of, 14
Butter fat as supplement to wheat kernel, 47
Butter fat, centrifuged, nitrogen content of, 114
Butter fat, centrifuged, phosphorus content of, 114
Butter fat, content of *A* in, 66, 67

Butter fat, extraction of *A* from by ether, 111
Butter fat in diet, 173
Butter fat, thermostability of *A* in, 90, 91
Butter fat, variation in quantity of *A* present in heated, 91
Butter, influence of storage on vitamin content of, 74
Butter-making, influence of method on vitamin content of product, 74
Butter oil as source of *A*, 136
Butter oil, deterioration of *A* on keeping, 90
Butter oil, phosphorus content of, 114
Butter pigment, relation to content of *A*, 75
Butter, seasonal variation of content of *A* in, 74

Cabbage, content of *A* in, 73, 136
Cabbage, content of *B* in, 81
Cabbage, antiscorbutic value of, 85, 232
Cabbage, dried, antiscorbutic value of, 100
Cabbage, dried, content of *A* in, 73
Cabbage, dried, content of *B* in, 81
Cabbage, dried, thermostability of *C* in, 100
Cabbage, effect of alkali on *B* in, 120
Cabbage, effect of storing on antiscorbutic value of, 105, 106
Cabbage juice, thermostability of *C* in, 100
Cabbage, thermostability of *A* in, 92
Cabbage, thermostability of *B* in, 96, 98
Cabbage, thermostability of *C* in, 99, 100, 185
Cages for rats, types of, 21, 22, 23
Calcium content of milk, effect of heat on, 281, 282, 283
Calcium deficiency a factor in rickets, 193, 195, 197, 199
Calcium depreciation a factor in pellagra, 245, 247
Calcium, importance in nutrition, 311
Calcium in dried milk, 287
Calcium in milk, effect of vitamin-free diet on, 274
Calcium, effect of cod-liver oil on retention of, 177, 178
Calcium lactate in basal diet, 103
Calcium metabolism in scurvy, 228, 229
Calcium metabolism, relation of vitamins to, 177
Calcification of bones, effect of cod-liver oil on, 177
Calcification of bones and teeth, relation of vitamins to, 177
Calorie, xi
Calorie theory, 1
Calorific value of food, xii
Calorimeter, xi
Calves, beriberi-like symptoms in, 165
Calves, effect of lack of vitamins on, 165
Calves, feeding experiments with, 165, 278, 283
Canned carrots, antiscorbutic value of, 101
Canned foods, content of *B* in, 98
Canned meats, antiscorbutic value of, 87
Canned spinach, variation in content of *C* in, 85
Canned string beans, content of *C* in, 85
Canning, antiscorbutic value of foods affected by, 99
Canning, effect on content of *A* in fruits and vegetables, 93, 94
Canning, effect on vitamin content of foods, 93, 94, 99, 307
Carbohydrates, ix, x
Carbohydrates, abnormal hydrolysis of, a cause of beriberi, 211
Carbohydrates, absorption of, x
Carbohydrates, digestion of, x
Carbohydrates a factor in pellagra, 247
Carbohydrate metabolism, relation of vitamins to, 162 (footnote), 172, 173, 179, 180, 211, 311
Carbon bisulphide, solubility of *A* in, 111
Cardiac enlargement, relation of vitamins to, 309
Cardio-respiratory phenomena in scurvy 218, 219
Carotin, association with *A*, 76, 112
Carotin content of ewe-milk fat, 115
Carotin-free basal rations, 115
Carotin in ewe-milk fat, 77
Carotin in pork liver, 77
Carotinoid-free diet, growth and reproduction on, 115
Carotinoid-free eggs, feeding experiments with, 77, 115
Carotinoid-free egg yolk as source of *A*, 77
Carotinoids, association with *A*, 76, 115
Carotinoids in pork liver, 76
Carotinoid-pigment, relation to vitamin *A*, 115
Carrots, canned, antiscorbutic value of, 101

Carrots, content of *A* in, 73, 75, 136
Carrot, content of *B* in, 81, 82, 151
Carrot, content of *C* in, 85
Carrots, cooked, antiscorbutic value of, 101
Carrots, effect of storing on antiscorbutic value of, 105
Carrots, extraction of *A* from by solvents, 111, 112
Carrots, thermostability of *A* in, 92
Carrots, thermostability of *B* in, 96
Carrots, thermostabiliy of *C* in, 101
Carrots, variation in nutritive value with age, 101
Casein, 54, 236
Casein an ingredient of experimental diets, 7, 10, 12, 14, 15, 57
Casein as supplement to oat kernel 49
Casein as supplement to wheat protein, 47, 49
Casein, bacterial growth-stimulant, 160
Casein, biologic value of, 266
Casein, effect of heat on (see caseinogen)
Casein, nutritive efficiency of, 55
Casein, purification of, 57, 58, 280, 281
Casein, remedial effect in pellagra, 259
Casein, sulphur content of, 280
Caseinogen, 262, 263
Caseinogen, effect of heat on, 280, 281
Catalase in tissues, reduction of, in polyneuritis, 187
Catalase, production of stimulated by *B*, 187
Catalysts, 5
Catalysts, vitamins as, 5, 185
Catalyzer, nickel, used in hydrogenation a factor in destruction of vitamins, 113
Cats, feeding experiments with, 18, 119, 194, 196 (footnote), 216 (footnote)
Cats, polyneuritis in, 119, 207
Celiac disease due to lack of vitamins, 172
Central nervous system, effect of lack of vitamins on, 171, 172
Cereal diet, xerophthalmia resulting from restriction to, 235
Cereal germ, content of *B* in, 78, 79
Cereal grains, dry, content of *C* in, 83
Cereal grains, relative values of proteins in, 55
Cereals, sprouted, antiscorbutic value of, 232
Cereals, sprouted, thermostability of *C* in, 100
Cereals, vitamin index of, 132
Cerebellar symptoms in avian polyneuritis, cause of prompt appearance of, 184
Cerebrosides in the diet, 15
Charcoal as absorbent of *B*, 117
Chard, content of *A* in, 72
Chard, thermostability of *A* in, 92
Cheese, vitamin content of, 302
Chemical properties of *B*, 130, 131
Chemical structure of vitamins, 128, 129
Chickens, experiments with carotinoid-free diet for, 76
Chickens, nutritive requirements of, 21
Chickens, synthesis of vitamins by, 167, 221
Chickens, vitality influenced by vitamin content of mother's diet, 88
Chickens, xerophthalmia in, 235
Children's diseases, relation to vitamin deficiency, 172, 292, 310, ff
Chinese, beriberi among, 201
Chlorine metabolism in scurvy, 228
Chloroform, solubility of *A* in, 111
Chloroform, solubility of *B* in from horseflesh in, 117
Chlorosis, deficiency of vitamins a factor in, 174
Chocolate yeast, 148
Chocolate, vitamin, 148
Cholesterol, 7, 8, 15, 112
Choline, 122, 123, 183
Choline-like bases from rice, antineuritic properties of, 127
Chronic polyneuritis, 208
Cider, antiscorbutic value of, 83, 229, 230
Cinchonine, antineuritic properties of, 128
Circulatory disturbances in latent scurvy, 218, 219
Circulatory disturbances, relation of vitamins to, 309
Citrazinic acid, 128
Citric acid, effect on thermostability of *C*, 100, 134, 135
Citrus juices, minimum antiscorbutic dose for guinea-pig, 232
Clover, content of *A* in, 72, 73, 136
Clover, content of *B* in, 81
Clover, dried, as source of *A*, 137
Cochlearia officinalis, 232
Cod liver oil, effect on calcification of bones, 177, 195
Cod liver oil, remedial effect in rickets, 194, 195, 198, 199
Cod liver oil, thermostability of *A* in, 90
Cod liver oil, vitamin content of, 68, 69

Colon, relation of vitamin deficiency to inflammation of, 311
Colon, effect of lack of *B* in, 173
Colostrum, content of *A* in, 74
Commercial vitamin preparations, 147, 148, 149
Complex of *A* existing in plant tissues, 111
Compound lard, 114
Concentration of *B* from animal tissue, method of Voegtlin, 138
Concentration of *B*, method of Funk, 139
Concentration of *B*, method of Gams, 140, 141
Concentration of *B* from pancreatic extract, 141, 142
Concentration of *B*, method of Fraenkel and Schwarz, 142
Concentration of *B* from wheat germ, 142, 143
Concentration of *B*, method of Bosshard and Hefti, 137, 138
Concentration of *C* from fruit juices, 143 and ff
Concentration of *C* from fruit juices, McClendon's method, 146, 147
Condensed milk, antiscortubic value of, 104, 165 (footnote)
Condensed milk in infant feeding, 295
Condensed milk, nutritive value of, 282, 283, 286
Condiments, 6
Congestion, intestinal, relation of vitamin deficiency to, 173, 311
Conjunctivitis, 234 and ff (See also Xerophthalmia)
Conjunctivitis, effect of yeast on, 311
Constipation, effect of yeast on, 311
Constipation, relation of scurvy to, 226, 227
Constipation, relation of vitamins to, 309
Consumption of food on deficient diets, 175
Control diets, 22
Convicts, feeding experiments to produce beriberi in, 203, 204
Cooked food, necessary data in experiments with, 89
Cooking, effect on antiscorbutic value of food, 232
Cooking, effect on antiscorbutic value of potatoes, 103
Cooking, effect on vitamin content of foods, 306, 307
Cooking of vegetables, effect of acids and alkalis in, 134, 135
Corn, biological value of protein of, 258
Corn embryo, antineuritic properties of, 78
Corn endosperm, antineuritic value of, 150
Corn gluten, lactalbumin as supplement to, 268
Corn in antiscorbutic ration, 83
Corn, kaffir, in antiscorbutic ration, 83
Corn meal, ether extraction of *A* from, 111, 112
Corn meal, nutritive deficiency of, 47
Corn, milk-producing value of, 265
Corn oil, relation between color and content of *A*, 76
Corn protein, nutritive value of, 47, 258
Corn, variation in content of *A* in, 75
Corn, vitamin index of, 80
Corn, white, content of *A* in, 75
Corn, yellow, thermostability of *A* in, 92
Corn, yellow, content of *A* in, 75
Correlation of vitamin content with function, 74, 75
Cortex, adrenal, degenerative changes caused by lack of *C*, 177
Cottonseed oil, relation between color and content of *A*, 76
Cotton seed, toxicity of, 32
Cows, effect of ration on vitamin content of milk, 165
Cows, feeding experiments with, 274, 275
Creatinine in milk, 262
Creatinuria on diets deficient in *B*, 180
Crisco in basal diet, 237
Criticism of vitamin research, 34
Crystalline preparations of antineuritic substances, 121 and ff
Cucurbitous vegetables, thermostability of *A* in, 92
Curd of milk, 263
Curve, time, of destruction of *C* in tomato juice, 102
"C-Y," 148
Cyclic compounds, influence of vitamins in syntheses of, 187
"Cyclokeiases," 187
Cystine, xi, 54
Cystine as supplement to casein, 54, 266
Cystine as supplement to lactalbumin, 267

Dairy products in the diet, dispensability of, 114
Dairy products in experimental diets, 114

Dandelion leaves, effect of drying on antiscorbutic value of, 105
Dandelion leaves, thermostability of *C* in, 135
Dasheen, content of *A* in, 73, 75
Dasheen, content of *B* in, 82
Demineralization of organism in pellagra, 245, 246
Demineralization of organism on scorbutic diet, 228, 229
Deficiency diseases in breast-fed children, 293
Deficiency of *A*, symptoms of, 167 and ff
Dehydration an unprofitable method of preserving vegetables, 106
Dehydration of foods by vacuum process, advantage of, 107
Desiccation, See Drying
Destruction of antiscorbutic vitamin by heat, factors influencing, 107, 108, 109
Dextrin in basal diets, 57
Dialysis of *B*, 117, 134
Dialysis of *C*, 132, 134
Diet, antineuritic, 215
Diet influencing vitamin content of milk, 74, 82
Diet, modified, for prevention of beriberi in Japanese navy, 203
Diet, neuritic, used at Kut-el-Amara, 206
Diet, neuritic, used in Japanese navy, 203
Diet, neuritic, used in Javanese prisons, 204
Diet, neuritic, used in lunatic asylum, 204
Diet, neuritic, used in Philippines, 205
Diet, sterilized, effect on human beings, 203, 204
Diets, beriberi-producing, 203, 204, 205, 206
Diets, control, 22
Diets deficient in fat, effect on children of, 235, 236
Diets in rachitic families, 195
Diets, Mellanby's rachitic, 196, 197, 198
Digestion, effect of lack of *B* on, 171, 172, 173
Digestion of foodstuffs, x
Digestive glands, function of vitamins in stimulating, 184 and ff
Dilation, intestinal, relation of vitamin deficiency to, 311
Diphtheria, relation of vitamin deficiency to, 310
Dogs, feeding experiments with, 97, 119, 167, 177, 178, 196, 197, 198, 199, 200, 253
Dogs, polyneuritis in, 207
Domestication theory of rickets, 195
Dosage of vitamins, methods of determining minimum, 34
Dosage of vitamins, standardization of, 131
Doves, sensitiveness to lack of *B*, 143 (footnote)
"Dry" beriberi, 206
Dried grasses as source of *A*, 137
Dried milk, antiscorbutic value of, 104
Dried milk, in infant feeding, 295
Dried milk, McClendon's preparation of, 147
Dried milk, nutritive value of, 286 and ff (See also Milk powder)
Dried plant products as source of *A*, 137
Dried potato, nutritive deficiencies of, 53
Drier for concentration of vitamins, McClendon's, 144, 145, 146
Drying, destruction of *C* in beef by, 109
Drying destruction of *C* in cabbage by, 100
Drying, destruction of *C* in potatoes by, 107
Drying, effect on antiscorbutic value of foods, 99, 105 and ff
Drying, effect on antiscorbutic properties of food, 232
Drying, effect on antiscorbutic value of tomatoes, 108
Drying, effect on antiscorbutic value of vegetables, 106
Drying, effect on content of *A* in foods, 93
Drying, effect on content of *B* in foods, 94, 96
Drying, effect on vitamin content of foods, 307, 308
Drying of fruit juices, commercial process for, 109
Drying of milk, commercial processes for, 146, 147, 286, 287
Drying of vegetables, optimum temperatures for, 107
Duodenal ulcer, effect of yeast on, 311
Duodenitis in beriberi, 206

Eczemas, effect of yeast on, 311
Edestin, bacterial growth-stimulant in, 160
Edestin, biologic value of, 266
Edestin, retention of *B* by, 130
Eel fat as cure for xerophthalmia, 235

Efficiency of animal body, ix
Eggs, antiscorbutic value of, 86
Eggs, antineuritic value of, 82
Eggs as supplement to wheat flour, 55
Eggs, carotinoid free, as source of *A*, 77
Eggs, content of *B* in, 82
Eggs, dried, as source of *B*, 81
Eggs, variation in antiscorbutic value due to diet of hens, 88
Eggs, vitamin content of, 302
Egg-yolk, antineuritic value of, 82, 215
Egg-yolk, antineuritic and growth-promoting power compared, 152
Egg-yolk an ingredient of experimental diets, 8, 12
Egg-yolk fat, vitamin content of, 68
Egg-yolk, growth-promoting value of, 3, 12, 15
Egg-yolk, ether extraction of *A* from, 111
Egg-yolk, ether extraction of *B* from, 116
Egg-yolk, thermostability of *A* in, 90
Elizabeth, N.J., outbreak of beriberi at, 206
Emaciation, lack of *A* a cause of, 167, 170
Emanation, radium, effect on *B* in yeast, 98
Embryo, animal, content of *B* in, 182
Embryo, corn, content of *B* in, 78
Embryo, wheat, content of *B* in, 78
Endocrine function, relation of vitamins to (See Internal secretion)
Endocrine glands, content of vitamin *B* in, 82, 185
Endocrine glands, relation of vitamin deficiency to disturbance of, 171, 172, 185, 186, 187, 311
Endosperm, corn, lack of *B* in, 150
Endosperm, distribution of vitamin *B* within, 79
Endosperm, grain, lack of *B* in, 78, 150
Endosperm of seeds, composition of, 75
Endosperm, wheat, as source of *B*, 78, 150
Energy of body, xi
Energy value of food, xi
Energy of muscular work, xii
Energy requirement, xiii
"Enriched" milk in infant feeding, 296
Ensiling, effect on content of *A* in fodder, 92
Enzymes, 185
Enzyme action a factor in the destruction of antiscorbutic power of orange juice, 109
Enzymes, effect on thermostability of antiscorbutic vitamin, 107, 108, 109
Erythema, effect of yeast on, 311
Ether, solubility of *A* in, 111, 112, 136
Ether, solubility of *B* in, 116, 117
Ethyl acetate, solubility of *B* from horse-flesh in, 117
Evaporated milk in infant feeding, 295
Evaporated milk, nutritive value of, 282, 283, 286
Ewe milk fat as food for rats, 77
Ewe milk fat as source of *A*, 115
Ewe milk fat, carotin content of, 77, 115
Exercise, beneficial effects in rickets, 196, 198
Exogenous hormones, 186
Experimental animals, advantage of small, 18
Experimental animals, considerations governing selection of, 44
Experimental animals, treatment of, 21, 22, 23, 24, 45
Extraction of *A* from plant tissues, 111, 112, 136
Extraction of *A* from plant tissues by solvents, factors influencing, 111, 112
Extraction of *A* from plant tissues, McClendon's method for, 137
Extraction of *A* from saponified butter fat, 113
Extraction of *B*, 137 and ff
Extracts of vitamins as supplement to diet, 297, 302, 308
Eye diseases due to lack of *A* (See Xerophthalmia)

Fat content of milk, variation in, 261, 262
Fat deficiency a factor in rickets, 194, 196, (footnote), 199
Fat free diets, experiments with, 163, 179, 194, 196 (footnote), 199, 236, 237
Fat metabolism, relation of vitamins to, 173, 179, 180
Fat-soluble vitamin, 4
Fat-solubility of *B*, 117
Fat starvation in children, 235, 236
Fats, ix, x
Fats, absorption of, x
Fats, antirachitic value of, 194, 196, 197, 198
Fats, digestion of, x
Fats, dispensability in diet, 179, 235, 236, 237
Fats, function in diet, 236, 237
Fats, hydrogenated, 301
Fats, vegetable, content of *A* in, 71
Fats, vegetable, in infant feeding, 296

Fats, vegetable, used in oleomargarine, 301
Fatty acids, x
Fatty infiltration due to lack of *B*, 173
Feces from oat diet, character of, 49
Ferric hydroxide, colloidal, as absorbent of *B*, 118
Fibrin, bacterial growth-stimulant in, 160
Fish, biological value of protein of, 258
Fish, content of *A* in, 71
Fish, content of *B* in, 82
Fish oils, vitamin content of, 68
"Flash" pasteurization, 277, 282
Flaxseed, content of *A* in, 73
Flaxseed protein, biological value of, 51, 52
Flavoring extracts, function in diet, 175
Flocculent precipitates as absorbents of *B*, 118
Flour, bolted, a cause of beriberi, 206
Flour milling, removal of vitamin in, 79
Flour, soya bean and wheat mixtures, 77
Flour, white, deficiency of, 79, 214
Folin-Macallum reagent, 131
Folliculitis, effect of yeast on, 311
Food consumption on deficient diets, 175
Food consumption, effect of milk on, 10 (See also Food intake)
Food intake, methods of determining, 22, 24, 33, 34
Food intake, necessity for data concerning, 34
Food preservation, effect on antiscorbutic value, 99
Foodstuffs, ix, x
Fourth vitamin, evidence for, 150 and ff
Fowls, feeding experiments with, 13, 25, 76, 88, 143 (footnote), 180, 183, 203, 205, 208, 216 (footnote), 235, 254
Fowls, vitamin *A* requirement of, 76
Fractionation of acetic acid solution of *B* by alcohol, 115, 116, 119
Frozen meat, antineuritic value of, 97
Frozen meat, antiscorbutic value of, 110
Fruit juices, commercial drying of, 109, 147
Fruit juices, concentration of *C* from, 144 and ff
Fruit juices, dried, stability of *C* in, 144 and ff
Fruits as antiscorbutics, 83
Fruits, content of *A* in, 74
Fruits, content of *B* in, 82
Fuller's earth, activated, 141, 154
Fuller's earth, activated, antineuritic and growth-promoting power compared, 154
Fuller's earth an absorbent of vitamins, 113, 117, 118
Function of organs correlated with vitamin content, 74, 75
Function, physiological, of vitamins, 181 and ff
Functioning cells, correlation of vitamin content with, 181, 182
Furunculosis, effect of yeast on, 311

Gastric disturbance in beriberi, 206
Gastric glands, stimulation by vitamins, 184
Gastric insufficiency, relation of vitamins to, 172
Gastrin, 186
Gastro-intestinal catarrh, effect of yeast on, 311
Gastro-intestinal disorders, lack of vitamins a cause of, 172, 173
Gastro-intestinal tract, effect of lack of vitamins on, 173
Gelatin as supplement to oat kernel, 49
Gelatin, bacterial growth-stimulant in, 160
Georgia velvet bean, content of *A* in, 73, 74 (footnote)
Georgia velvet bean, thermostability of *A* in, 92
Germ (See also Embryo)
Germ, commercial wheat, as source of antineuritic, 215
Germ of seeds, composition of, 75
Germ, wheat, growth promoting and antineuritic power compared, 152
Germinating lentils, antiscorbutic value of, 84
Germinating peas, antiscorbutic value of, 84
Germinating seeds, antiscorbutic value of, 84
Germination of seeds for use as antiscorbutics, 84
Glands, adrenal, effect of lack of *C* on, 177
Glands, intestinal, relation of vitamin deficiency to depressed activity of, 311
Glands, lymph, content of *B* in, 82
Glands of internal secretion (See Endocrine glands)
Glands, stimulation by vitamins, 184, 185, 186, 187
Glands, swollen, effect of yeast on, 311

Glandular adipose tissue, effect of vitamin deficiency on, 180
Glandular organs, value in the diet, 303
Glandular organs, vitamin content of, 303
Glucose, stimulating effect on yeast growth, 41, 42
Glucosides, hormones produced from, 186
Gluten feed, milk-producing value of, 265
Gluten feed, nutritive deficiency of, 47
Glyoxalase content of liver in polyneuritis, 187
Goats, feeding experiments with, 274, 276
Gonococcus, 317
Grains, distillers, milk-producing value of, 265
Grains, thermostability of *A* in, 92
Grains, thermostability of *B* in, 96
Grape-fruit, content of *A* in, 74
Grape-fruit, content of *B* in, 82
Grape-fruit juice, dried, antiscorbutic value of, 109
Grape-juice, antiscorbutic value of, 83
Grape-juice, content of *B* in, 82
Grasses as source of *A*, 72, 73, 136, 137
Grasses as source of *B*, 81
Gravimetric yeast test for *B*, 37
Grip infection, relation of vitamin deficiency to, 310
Ground nut (See Peanut)
Growth and appetite, relation between, 175, 176
Growth, effect of *A* on, 167, 168
Growth, effect of *B* on, 175
Growth inhibition on diet containing vitamin, 152
Growth promoting and antineuritic factors, comparison of thermostability of, 154 and ff
Growth-promoting as distinct from antineuritic factor, 150 and ff
Growth-promoting as distinct from antineuritic factor, distribution of, 150
Growth-promoting factor, properties of, 153 and ff
Growth promoting factor, separation from antineuritic, 153, 154, 159, 160
Growth-promoting factor from yeast, separation of, 152, 153
Growth-promoting function of *A*, 168
Growth-promoting value of brewer's yeast, 14
Growth-promoting value of butter-fat, 3, 12
Growth-promoting value of egg-yolk, 3, 12, 15
Growth-promoting value of meat, 197
Growth-promoting value of milk, 3, 9, 10, 11
Growth-promoting value of protein, numerical expression for, 55
Growth stimulation in children due to administration of vitamin, 296, 297
Guinea-pig, requirements for *C*, 166, 232, 233
Guinea-pigs, feeding experiments with, 18, 19, 25, 33, 83, 84, 85, 86, 99, 100, 101, 102, (footnote), 103, 104, 105, 133, 147, 166, 178, 269, 271, 277, 284, 290
Guinea-pigs, symptoms of scurvy in, 221, 222, 223
Guinea-pig test for presence of *C*, value of, 232, 233

Hay, antiscorbutic value of, 104, 105, 290
Hay from immature grasses, content of *B* in, 81
Heart action, effect of vitamin extract on, 183
Heart, effect of lack of *A* on, 169
Heart, effect of lack of *B* on, 171, 173
Heart muscle, effect of lack of *B* on, 173
Heart, effect of lack of *C* on, 228 (footnote)
Heart, ox, antineuritic and growth-promoting power compared, 152
Heart, ox, bacterial growth-stimulant in, 160
Heart, pig, dried, content of *A* in, 91, 92
Heart protein, content of *B* in, 82
Heat, factors influencing destruction of *B* by, 120
Heat, factors influencing destruction of *C* by, 107, 108, 109
Heat, stability of *A* to, 90, 91
Heat, stability of *B* toward, 94, 119, 120, 155 and ff
Heat, stability of *C* to, 99, 100, 107, 133, 134, 135, 284, 285
Heating, effect on antiscorbutic properties of food, 232
Heating, effect on nutritive value of milk, 277 and ff
Hemicelluloses interfering with extraction of *A* from plant tissues, 112
Hemorrhage, intestinal, relation of vitamin deficiency to, 311

Heredity in rickets, 193
Hippuric acid, excretion of by pellagrins, 246
Histidine, 122, 126, 259
Histological changes of ribjunction due to lack of *A*, 99
Historical development of vitamin theory, 6
"Hold" pasteurization, 278, 282
Hormones, 6, 185, 186, 187
Horse-flesh, antineuritic substance from, 117
Hydrochloric acid, administration to pellagrins of, 258
Hydrochloric acid, alcoholic, as extractive for *B*, 118
Hydrochloric acid, inactivation of *B* by, 118
Hydrogen ion concentration of foods in relation to antiscorbutic power, 133, 134
Hydrogen ion concentration, influence on thermostability of *C* in tomato juice, 102 (footnote)
Hydrogen peroxide, destruction of *C* by, 133
Hydrogenated fats in diet, use of, 301
Hydrogenated oils in basal diets, 58
Hydrogenation, effect on vitamin content of oils, 90, 113
Hydrolysis, acid, effect on rice polishings, 118, 119
Hydrolysis of carbohydrates, beriberi caused by abnormal, 211
Hydroxyl ion, effect on *C*, 134
Hydroxy-nicotinic acid, antineuritic properties of, 128
Hydroxy-pyridines, antineuritic properties of, 129, 182
Hydroxy-pyridine glutazine, 128
Hydroxy-pyrimidines, 127, 128
Hyperadrenalinemia due to lack of *B*, 171, 172

Ilium, effect of lack of *B* on, 173
Inactivation of *B* by acids, 118
Inactivation of vitamins, theory of, 129
Inanition, chronic, due to lack of *B*, 171, 311
Index of cereals, vitamin, 132
Indican in beriberi, 172
"Indicator limit" of phosphorus pentoxide, 80
Indole derivatives, antineuritic properties of, 182
Infantile scurvy, 219, 220
Infantile scurvy, secondary factors in, 285
Infection and nutritional disorders, relation between, 310
Infection, bacterial, relation of vitamin deficiency to, 311
Infection, relation between vitamin deficiency and susceptibility to, 167, 172, 173
Infection, relation of vitamin deficiency and susceptibility to, 310
Infection, susceptibility of scorbutic animals to, 219
Infection theory of beriberi, 202, 209, 210
Infection theory of pellagra, 245 (footnote), 247, 248, 260
Infection theory of rickets, 193
Infection theory of xerophthalmia, 237, 238, 239
Infiltration, fatty, due to lack of *B*, 173
Influence of diet on vitamin-content of body fats, 70
Influenza bacillus, 317, 318
Infusorial earth as absorbent of *B*, 118
Inorganic elements in nutrition, study of, 64
Inorganic salts as supplement to wheat kernel, 47
Inorganic salt mixtures in experimental diets, 10, 11, 12, 23, 24
Inorganic salts of milk, 268, 274
Internal secretion, influence of vitamin, on, 6, 7, 162 (footnote), 171, 185, 186, 187
Internal secretion, rickets due to deficiency of, 193
Intestinal congestion, relation of vitamin deficiency to, 311
Intestinal dilation, relation of vitamin deficiency to, 311
Intestinal hemorrhage, relation of vitamin deficiency to, 311
Intestinal insufficiency, relation of vitamin deficiency to, 172
Intestinal intoxication, effect of yeast on, 311
Intestinal muscles, effect of vitamin extracts on, 183
Intestinal putrefaction, abnormal, in beriberi, 173
Intestinal stasis, relation of vitamin deficiency to, 172, 311
Intestinal symptoms of deficiency of *A*, 168
Intoxication a cause of rickets, 193

Intoxication theory of beriberi, 210, 211
Intoxication theory of pellagra, 247
Intraperitoneal injections in polyneuritis, 117
Intravenous administration of vitamins, 184
Intravenous injection of orange juice, effect of, 226
Intussusception, relation of vitamin deficiency to, 311
Invertase activity of yeast, stimulant for, 160 (footnote)
Invertase as factor influencing thermostability of *C* in orange juice, 109
Isolation of *B*, 121, ff
Isolation of *B*, method of Edie et al, 122
Isolation of *B*, method of Funk, 121, 122
Isolation of *B*, method of Myers and Voegtlin, 126
Isolation of *B*, method of Pol, 124, 125
Isolation of *B*, method of Seidell, 127
Isolation of *B*, method of Suzuki et al, 123
Isomeric hydroxypyridines, 128, 129
Isomerization, loss of antineuritic power from, 128, 129

Japanese children, xerophthalmia, in, 235
Japanese navy, study of beriberi in, 202, 203
Javanese prisons, beriberi-producing diet of, 204
Just-Hatmaker process for preparation of dried milk, 286, 287

Kaffir battalion, outbreak of scurvy in, 84
"Kalzoze," 17
Kaolin as absorbent of vitamin *B*, 117
Katjang idjo, 124
Katjang idjo, separation of *B*, from, 124, 125
Katjang idjo, thermostability of *B* in, 94
Keratitis, 169, 237
Keratomalacia, 234 and ff (See also Xerophthalmia)
Kidney, effect of lack of *A* on, 169
Kidney, effect of lack of *B* on, 171, 173
Kidney, effect of lack of *C* on, 228 (footnote)
Kidney, pig, extraction of *B* from, 117
Kidney protein, content of *B* in, 82
Kidney, vitamin content of, 182
Kieselguhr, absorption of yeast vitamin by, 118
"Krystalak," 236
Kut-el-Amara, outbreak of beriberi at, 206

Labrador, outbreak of beriberi in, 206
Lactalbumin, 54, 262, 263
Lactalbumin an incomplete protein, 267 268
Lactalbumin and lactose, supplementary relation between, 267
Lactalbumin as supplement for maize protein, 54, 268
Lactalbumin, biologic value of, 55, 266, 267, 268
Lactalbumin in the digestive tract, process of hydrolysis of, 267, 268
Lactation, diet during, 272, 273, 294, 295
Lactation, underfeeeding cause of failure in, 272, 273
Lactation, vitamin requirements during, 294, 295
Lactoglobulin, 262, 264
Lactose an ingredient of experimental diets, 15, 57
Lactose and lactalbumin, supplementary relation between, 267
Lactose compared with cane sugar, physiological efficiency of, 262
Lactose, effect of heat on, 280
Lactose in milk, 262
Lard, digestibility of, 69
Lard manufacture as influencing vitamin content of product, 70, 71
Lard substitutes, digestibility of, 69, 70
Lard substitutes, vitamin content of, 113
Lard, vitamin content of, 69
Laxative diets, scurvy produced on, 227
Leaf, amino-acid content of, 52
Leaf and seed mixtures, biological value of, 52
Leaf as supplement to food in diet, 52
Leaf, biological value of, 52
Leaf, mineral content of, 52
Leaf, protein content of, 52
Leaf tissue, thermostability of *A* in, 92
Leafy vegetables, antiscorbutic value of, 85, 232
Leaves, content of *A* in, 72, 73, 74
Leaves, extraction of *A* from, 77
Leaves, green, synthesis of *A* by, 77
Lecithalbumin, 264
Lecithin, 7, 8, 15, 112
Legume and cereal mixtures, biological value of, 50
Lemon juice as antiscorbutic, 83

Lemon juice as antiscorbutic in the navy, 230
Lemon juice as antiscorbutic for infants, 298
Lemon juice, dried, antiscorbutic value of, 109
Lemon juice, influence of storage on antiscorbutic value of, 108
Lemon juice, minimum antiscorbutic dose for guinea-pig, 232
Lemon juice, thermostability of *C* in, 103
Lemons, content of *A* in, 74
Lemons, content of *B* in, 82
Lemons, antiscorbutic value of, 229, 230
Lentils, antineuritic value of, 77, 82, 215
Lentils, extraction by water of *B* from, 115
Lentils, germinated, thermostability of *C* in, 100
Lentils, germinating, antiscorbutic value of, 84
Lentils, growth-promoting power compared, 152
Lettuce, content of *A* in, 72
Lenco compound of yellow pigment, hypothesis of, 76
Lime juice as antiscorbutic, 83, 232
Lime juice, isolation of *B* from, 122
Lime salts, effects on rickets, 64, 65
Linseed oil, antirachitic value of, 196, 197
Lion cubs, feeding experiments with, 194
Lipoids, association of *B* with, 131
Lipoids, dispensability of, 8
Lipochromes, 76, 112
Lipochromes, association with vitamin *A*, 76
Lipochrome-like substances in pork liver, 77
Liver as cure for xerophthalmia, 235
Liver, effect of lack of *A* on, 169
Liver, effect of lack of *B* on, 171, 173
Liver, ox, antineuritic value of, 82
Liver, pig, dried, content of *A* in, 91
Liver, pork, carotinoids in, 76, 77
Liver, rat, as antiscorbutic, 166
Liver, seal, as antiscorbutic, 230
Liver tissue, content of *B* in, 82
Liver, vitamin content of, 182
Lloyd's reagent, 118, 142, 153
Lloyd's reagent, "activated," 142
Lloyd's reagent as absorbent of *B*, 118
Long-continued experiments, necessity for, 26, 27, 45
Lozenges, antiscorbutic, of Bassett-Smith, 144, 145
Lukuga, beriberi in, 209
Lungs, effect of lack of *A* on, 169
Lungs, effect of lack of *B* on, 173
Lymph glands, vitamin content of, 82, 182
Lymphocytes, effect of vitamin deficiency on, 183
Lymphoid tissue, effect of vitamin deficiency on, 183
Lymphoid tissue, importance of, 183
Lysine, xi, 54, 55, 258, 259

Magnesium, toxicity of, 32
Maintenance and growth, vitamin requirements for, 162, 163
Maintenance requirement and appetite, relation between, 176
Maintenance requirement as distinguished from growth-requirement, 162
Maintenance requirements of different species, 165, 166
Maintenance requirement, vitamins in, 162, ff
Maize, biological value of protein of, 258
Maize, nutritive deficiencies of, 49, 55
Maize protein, amino acids of, 55
Maize protein, biological value of, 51
Maize, thermostability of *B* in, 95
Malnutrition, 300, 310
Malnutrition, administration of *B* in, 297
Malnutrition of nervous system, 171
Malnutrition, relation between deficiency of *A* and, 295
Malt, antiscorbutic value of, 84
Malt extract, antirachitic value of, 196
Man, susceptibility to lack of antiscorbutic, 166
Mangel, content of *A* in, 73, 75
Mangel, content of *B* in, 82
"Marmite," 215
Mastic as absorbent of *B*, 117
"Matok" as antiscorbutic, 230
Meat, alcohol extraction of *B* from, 115
Meat, antirachitic value of, 197
Meat, antiscorbutic value of, 86, 87, 228, 232
Meat as supplement to wheat flour, 55
Meat, biological value of protein of, 258
Meat, cooked, vitamin content of, 303
Meat, content of *A* in, 71
Meat, content of *B* in, 119
Meat, effect of alkali on *B* in, 119
Meat, ether extraction of *B* from, 116
Meat, extraction of *B* from, 115
Meat extracts, antiscorbutic value of, 88
Meat, frozen, antineuritic value of, 97

Meat, frozen, antiscorbutic value of, 88, 110
Meat, growth-promoting value of, 197
Meat juice, antiscorbutic value of, 88
Meat, preserved, antiscorbutic value of, 87
Meat, raw, antineuritic value of, 97
Meat, salted, antineuritic value of, 97
Meat, sterilized, deficiency in antineuritic power, 97
Meat, stimulating effect of, 197
Meat, thermostability of *B* in, 97
Meat, thermostability of *C* in, 110
Meat, tinned, antiscorbutic value of, 87
Medulla, adrenal, degeneration due to lack of *C*, 177
Meningococcus, growth-stimulant for, 317
Merrell-Soule process for drying fruit juices, 147
Merrell-Soule process for drying milk, 287
Metabolism, basal, xiii
Metabolism, calcium in scurvy, 228, 229
Metabolism, calcium, relation of vitamins to, 177
Metabolism, chlorine, in scurvy, 228
Metabolism, fat, relation of vitamins to, 179, 180
Metabolism, function of hormones in, 186
Metabolism in pellagra, 245, 246
Metabolism, nitrogen, effect of lack of *B* on, 184
Metabolism, nitrogen, relation of vitamins to, 180
Metabolism of nerve tissues, relation of *B* to, 183
Mabolism, phosphorus, relation of vitamins to, 179
Metabolism, protein, in scurvy, 218
Metabolism, relation between appetite and, 176
"Metagen," 149
Methods of concentration of vitamin *B*, 137 and ff
Methyl alcohol, solubility of *B* in, 116
Mice, feeding experiments with, 2, 6, 8, 14, 16, 17, 18, 63, 181, 305 (footnote)
Milk, albumin in dried, 287
Milk an ingredient of experimental diets, 3, 6, 8, 9, 10, 11, 47, 86, 104
Milk, antineuritic value of, 269
Milk, antiscorbutic value of, 86, 232, 277
Milk as supplement to cereal grains 265, 266
Milk as supplement to oats and bran in guinea-pig rations, 86
Milk as supplement to wheat flour, 55
Milk, autoclaved, as constitutent of scorbutic diet, 104
Milk, biological value of protein of, 258
Milk, boiled, digestibility of, 279
Milk, boiled, nutritive value of, 279
Milk, breast, of pellagrins, 246
Milk, breast, of women with beriberi, 202
Milk, calcium in dried, 287
Milk, caseinogen in dried, 287
Milk, composition of human compared with cow's, 261 and ff
Milk, condensed, antiscorbutic value of, 104, 165 (footnote)
Milk, condensed, in infant feeding, 295
Milk, condensed, nutritive value of, 282, 283, 286
Milk, condensing of, 165 (footnote)
Milk, constituents of, 261
Milk, content of *B* in, 81, 82, 151, 269, 297
Milk, content of *C* in, 269, 270
Milk, cow's, in infant feeding, 295
Milk diet, growth on, 270, 271
Milk, dried, antiscorbutic value of, 104 271, 288, 290, 291
Milk, dried, in infant feeding, 295
Milk, dried, vitamin content of, 288
Milk drying, Just-Hatmaker process 286, 287
Milk drying, McClendon's method, 146 147
Milk drying, Merrell-Soule process, 287
Milk, effect of diet of lactating animal on composition of, 164, 165, 272, 293, 294
Milk, effect of diet of lactating animal on nutritive value of, 273, 274, 275, 276, 293, 294
Milk, effect of diet of lactating animal on vitamin content of, 82, 164, 165
Milk, effect of heat on nutritive value of, 277 and ff
Milk, effect of heat on phosphorus content of, 283
Milk, effect of heat on vitamin content, 283
Milk, effect of small quantities in the diet, 3
Milk, evaporated, in infant feeding, 295
Milk, evaporated, nutritive value of, 282, 286
Milk, factors influencing content of *A* in, 74

Milk fat, composition of, 262
Milk fat, effect of diet of lactating animal on, 273, 274
Milk, goat, composition of, 261
Milk, globulin in dried, 287
Milk, human, caseinogen in, 263
Milk, human, salt content of, 268
Milk, human, fat content of, 262
Milk, influence of diet of lactating animal on antiscorbutic value, 86
Milk in infant feeding, comparison of raw and pasteurized, 279
Milk, inorganic salts in, 268, 274
Milk, isolation of *B* from, 122
Milk, lactose content of, 262
Milk, loss of *C* with aging of, 284
Milk of different species, composition of, 261 and ff
Milk, pasteurized, 277, 278
Milk, pasteurized, as infant food, 285
Milk powder, antiscorbutic value of, 104
Milk powder compared with fluid milk as sole diet, 270, 271
Milk powder, effect of heat on, 280
Milk powders, variations in, 291
Milk production, effect of quantity of food on, 272
Milk production, value of different protein feeds for, 265
Milk proteins, 262 and ff
Milk protein, biological value of, 51
Milk protein, effect of diet of lactating animal on, 274
Milk protein, relation of intake to growth, 47, 48
Milk proteins, value for milk production, 265
Milk ration required for children, 300
Milk, raw, antiscorbutic value of, 104
Milk, reduction of calcium content on heating, 281, 282, 283
Milk, scalded, antiscorbutic value of, 105
Milk, seasonal variation in vitamin content of, 276, 277
Milk, sheep, composition of, 261
Milk, skimmed, as source of vitamins, 301
Milk, solids in dried, 287
Milk, source of vitamins of, 275, 276
Milk, sterilized, containing vitamins, 278, 280
Milk sugar 262 (See lactose)
Milk, thermostability of *B* in, 96, 97
Milk, thermostability of *C* in, 104, 105, 284, 285
Milk, vitamin content of, 161, 269 and ff
Millet protein, biological value of, 51, 52
Millet seed, content of vitamin *A* in, 73
Milling of wheat, 214
Milling of rice, 212
Mineral content of the diet as furnished by seeds, 52
Mineral content of diet, influence on growth and reproduction, 48
Mineral content of milk, 268, 274, 275
Mineral content of seed and tuber mixtures, 53
Mineral deficiency as cause of deficiency diseases, 59 (footnote)
Mineral elements, role in body, x
Mineral elements, source of in diet, x
Mineral metabolism, 59 (footnote)
Mineral metabolism of pellagrins, 245, 246
Mineral oil added to scorbutic diets, 226
Mineral salts, ix, x
Mineral salts, 59 and ff
Mineral salts, rachitic-like condition produced by deficiency of, 199
Minimum dosage of vitamins, methods of determining, 33
Minimum dosage of vitamins, variation in, 30, 31
Minimum vitamin requirements, 163
McClendon's drier for concentration of vitamins, 144, 145, 146
McClendon's method for extraction of *A* from plant tissues, 137
"Modified milk" vitamin deficiency of, 297
Monkeys, feeding experiments with, 18, 86, 105, 170, 173, 179, 229, 254, 257 259, 269, 270, 284, 290, 311
Monkeys, polyneuritis in, 207, 208
Monkey, susceptability to lack of antiscorbutic, 166
Monotonous diets, nutritive value of, 8
Motor cells of cord, degeneration in polyneuritis, 130, 183
Mucous disease, relation of vitamin deficiency to, 172, 310
Muscle, beef, antineuritic value of, 82
Muscle, fish, antineuritic value of, 82
Muscle, heart, effect of lack of *B* on, 173
Muscle tone, regulation by vitamins of, 182, 183
Muscular tissue, effect of lack of vitamins on, 172
Mushrooms, failure to synthesize vitamin *A*, 77
Myetin, 262
Myenteron, relation of vitamin deficiency to atrophy of, 311

Nageli's solution, 35, 39
Nerve cells, effect of lack of vitamins on, 172
Nerve fibers, effect of lack of *B* on, 183
Nervous system, central, effect of lack of vitamins on, 171, 172
Neurasthenia, lack of vitamins a factor in, 174
Neuritis, 171, 309
Neuritis, relation of vitamins to, 309
Neuro-muscular mechanism, effect of lack of vitamins on, 172, 173
"Neutral lard," 301
Newfoundland, outbreaks in beriberi in, 206
Nickel, possible effect on vitamins, 113
Nicotinic acid, 122, 123, 127, 128, 182
Nicotinic acid, antineuritic properties of, 127, 182
Nicotinic acid, vitamin *B* a derivative of, 128
"Night-blindness," 241, 309
Nitrogen as a constituent of *A*, 114
Nitrogen as constituent of *B*, 128 (footnote), 132
Nitrogen balance of pellagrins, 245
Nitrogen balance on diets deficient in vitamins, 180
Nitrogen content of centrifuged butter fat, 114
Nitrogen metabolism, effect of lack of *B* on, 184
Nitrogen metabolism, relation between vitamins and, 180
Nitrogen of blood, effect of lack of *B* on, 174
Nitrous acid, inactivation of *B* by, 118
Nomenclature of vitamins, 4
Norit, 159, 160
Normality, criteria of, 25, 26
Nuclear fecundation a junction of vitamins, 181
Nuclear material, correlation of vitamin content with, 182
Nuclear starvation, lack of vitamins a cause of, 181
Nuclear tissues, content of *B* in, 82
Nucleate, sodium, an ingredient of synthetic diets, 7
Nucleic acid, 127, 187
"Nucleus" of vitamins, 129
Nutramine, 215 (footnote)
Nutritive requirements of different species, 20, 21
Nuts, content of vitamin *A* in, 74
Oat kernel, nutritive deficiencies of, 49, 51
Oat kernel, proteins of, 47, 49, 51, 55
Oatmeal, antineuritic value of, 215
Oat protein, amino acids of, 55
Oat protein, biological value of, 47, 51
Oedema, 169, 170, 171, 172
Oedema, relation between adrenals and, 171, 172
Oedema, relation between vitamins and, 169, 170
Oidium lactis, relation of vitamins to growth of, 316
Oil meal, milk-producing value of, 265
Oils, vegetable, content of *A* in, 71
Oleic acid, solubility of *B* in, 117
Oleomargarine, 114, 301, 302
Oleomargarine compared with butter as a constituent of the diet, 114
Oleomargarine, digestibility of, 301, 302
Oleomargarine, vitamin content of, 67, 302
"Oleo oil," 71, 75, 301
Oleo oil, vitamin content of, 71, 75
Oleo-stearin, vitamin content of, 71
Olive oil, solubility of *B* in, 117
Onion, content of *C* in, 85, 86
Onion, content of *B* in, 81, 150
Optics, effect of lack of *B* on, 173
Orange juice, antirachitic value of, 197
Orange juice, antiscorbutic value for infants, 298
Orange juice, dried, antiscorbutic value of, 108, 109
Orange juice, effect of intravenous injections of, 226
Orange juice, effect of storage on antiscorbutic value of, 108, 109
Orange juice, enzyme action a factor in destruction of antiscorbutic power of, 109
Orange juice, minimum antiscorbutic dose for guinea pig, 232
Orange juice, thermostability of *C* in, 103, 104, 109, 133
Orange peel as source of *B*, 171 (footnote)
Oranges, antiscorbutic value of, 83, 229, 230, 232, 298
Oranges, content of *A* in, 74, 197
Oranges, content of *B* in, 82, 151, 171 (footnote)
Organic foodstuffs, ix, x
"Oriflamma," 217
Oridine, 125
Orycinin, bacterial growth-stimulant in, 160

Orypan, 311
Oryzanin, 123, 124
Osteogenesis in rats on diets deficient in *A*, 170
Osteomalacia, acidosis a factor in, 193
Ovary, effect of lack of *B* on, 171, 173, 174
Ovary, storage of vitamins in, 164
Oxidation a factor in the destruction of *A*, 94
Oxidation a factor in the destruction of *C*, 100, 103, 133, 285
Oxidation a factor in the deterioration of heated milk, 285
Oxidation processes, relation of antineuritic vitamin to, 187
Oxygen, deterioration of *A* in oil through contact with, 90

"Paddy" rice, 212
Palatability a factor in nutrition, 7
Palm oil, destruction of *A* by steam, 92
Pancreas, content of *B* in, 82
Pancreas, effect of lack of *B* on, 171, 173
Pancreatic extract, separation of *B* from, 141, 142
Pancreatic insufficiency, relation of vitamins to, 172
Pancreatic juice, stimulation of flow by, 186
Pancreatic secretin, resemblance of *B* to, 186
Paper pulp as roughage, 103
Paralytic symptoms in polyneuritis, cause of, 172
Pasteurization of milk, 277, 278, 282
Pasteurization, effect on antiscorbutic value of milk, 284, 285
Pasteurized milk as infant food, 285
Peanut butter as medium for administration of vitamins, 148
Peanut, content of *A*, in, 74
Peanut meal, antineuritic value of, 215
Pea protein, biological value of, 50, 51, 52
Pears, content of *B* in, 82, 171 (footnote)
Peas, antineuritic value of, 77, 82, 215
Peas, antiscorbutic value of, 232
Peas as food for rats, 49, 50
Peas, dried, content of *B* in, 77
Peas, dried, thermostability of *B* in, 95
Peas, germinated, antiscorbutic value of, 84
Peas, germinated, thermostability of *C* in, 100
Peas, relation between color and content of vitamin *A*, 76
Pectins interfering with extraction of *A* from plant tissues, 112
Pellagra a communicable disease, 248, 249
Pellagra, age of susceptibility to, 243
Pellarga among Turks in Egypt, 244, 257
Pellagra, dietary hypothesis of, 249 and ff
Pellagra-producing diets, 249 and ff
Pellagra, etiology of, 120, 242 (footnote), 245 (footnote), 247 and ff
Pellagra, extent in U.S.A., 242
Pellagra, feeding experiments in, 252, 253, 254
Pellagra, geographic distribution of, 242, 243
Pellagra, medication as compared with dietary treatment for, 255
Pellegra, periodicity of, 243
Pellegra, relation of maize diet to, 242 (footnote), 247, 259
Pellagra, remedial diets for, 254, 255
Pellegra, susceptibility of men and women, 243
Pellagra, susceptibility of negroes and whites, 243
Pellegra, symptoms of, 243 and ff
Pellagra, vitamin deficiency a factor in, 250, 256, 257
Penicillium glaucum, relation of vitamins to growth of, 315
Peptone, bacterial growth stimulant in, 160
Petroleum ether, solubility of *A* in, 112
Philippine scouts, study of beriberi among, 205
Phosphates, effect on calcium metabolism in rickets, 65
Phosphatides, 112
Phosphoric acid in urine of beriberi patients, 211
Phosphoric anhydride as indicator of vitamin efficiency of grains, 79, 80
Phosphorus deficiency cause of rachitic like condition, 65
Phosphorus compounds, association of *B* with, 131
Phosphorus content of *A*, 114
Phosphorus content of *B*, 132
Phosphorus content of butter oil, 114
Phosphorus content of caseinogen, 263
Phosphorus content of centrifuged butter fat, 114
Phosphorus content of cereals a vitamin index, 132
Phosphorus content of lactalbumin, 263
Phosphorus content of milk, effect of heat on, 283

Phosphorus, effect of cod liver oil on retention of, 177, 178
Phosphorus, in cereals, relation between vitamin content and, 79, 80
Phosphorus in milk, effect of vitamin-free diet on, 274
Phosphorus metabolism in beriberi, 211
Phosphorus metabolism in scurvy, 228
Phosphorus metabolism, relation of vitamins to, 179
Phosphorus, remedial value in rickets, 64, 65, 199
Physiological function of vitamins, 181 and ff
Pickling, antiscorbutic value of foods affected by, 99
Pigeons, feeding experiments with, 3, 7, 14, 18, 96, 98, 102, 117, 123, 125, 143 (footnote), 147, 150, 152, 158, 159, 160, 162 (footnote), 163, 172, 179, 184, 204, 215 (footnote), 216 (footnote).
Pigeons, vitamin requirements of, 165
Pigment in butter fat, relation of vitamin content and, 67
Pigment, yellow, in peas, 76
Pigments, hypothesis of leuco compounds of, 76
Pigments, plant, correlation with vitamin *A* content, 75
Pilocarpine, 128, 182, 183
Pilocarpine, antineuritic properties of, 128, 182
Pine needles, cure of scurvy by decoction of, 217
Pituitary, effect of lack of *B* on, 171
Plant pigments, correlation with content of *A*, 75
Plant structures, relative content of *A* in, 72
Plant structures, relative content of *B* in, 80, 81, 82
Plant tissues, complex of *A* existing in, 111
Plant tissues, content of *B* in, 80, 81, 82
Plant tissues, dried, as source of *A*, 136, 137
Plant tissues, extraction of *A* from, 111, 112, 136
Plants, hormones in, 186
Plexus, Auerbach's, relation of vitamin deficiency to degeneration of, 311
"Polished" rice, 212
Polyneuritis, 13, 14
Polyneuritis, cause of paralytic symptoms in, 172
Polyneuritis, chronic, 208, 209
Polyneuritis, degeneration of sciatic nerve in, 150
Polyneuritis, degeneration of motor cells of cord in, 130, 183
Polyneuritis developed on diets containing excess of vitamins, 184
Polyneuritis gallinarium, 203
Polyneuritis identical with beriberi, 203, 204
Polyneuritis, respiratory quotient in, 216 (footnote)
Polyneuritis, symptoms in birds, 207, 208
Polyneuritis, symptoms in cats and dogs, 208
Polyneuritis, time of onset in birds, 205
Potassium hydroxide, alcoholic, effect on extract of *A*, 112, 113
Potassium metabisulphite as preservative for citrus fruit juices, 108 (footnote)
Potato, biological value of, 52, 53
Potato, content of vitamin *B* in, 81, 82, 96, 151
Potato, dried, commercial, content of *B*, 96
Potato, mineral content of, 52, 53
Potato peel, content of *B* in, 81
Potato supplemented by seeds, nutritive value of, 53
Potato, thermostability of *B* in, 96
Potatoes, antineuritic value of, 82, 151
Potatoes, antiscorbutic value of, 85, 102, 103, 135, 230, 298
Potatoes as antiscorbutic for infants, 298
Potatoes, content of *A*, 73, 75
Potatoes, cooked, antiscorbutic value of, 85, 102, 103
Potatoes, effect of acid on stability of *C* in, 135
Potatoes, effect of storing on antiscorbutic value of, 105
Potatoes, sweet, content of *A* in, 75
Potatoes, sweet, content of *B* in, 82
Potatoes, sweet, thermostability of *A* in, 92
Prairie dog, antiscorbutic requirement of, 166, 220
Preservation of foods, effect on vitamin content, 99, 307
Preservatives for *C* in citrus fruit juices, 103, 108 (footnote)
Preserved food, commercial uselessness of data concerning, 89
Properties, chemical, of *B*, 130, 131
Properties of *C*, 132

Protein a factor in pellagra, 257 and ff
Protein-free milk, 9, 10, 11, 14, 60, 61, 62, 161, 266, 267
Protein-free milk as source of *B*, 269
Protein-free milk, effect of heat on, 98, 280
Protein-free milk, nitrogen content of, 60, 61, 62, 267, 268
Protein-free milk, preparation of, 60, 61, 62
Protein-free milk, sulphur content of, 268
Protein intake, influence on growth, 47
Protein in the diet, relation between vitamins and, 173, 179, 180
Protein metabolism in scurvy, 218
Protein, numerical expression for growth-promoting value of, 55
Protein, requirement in diet, x
Proteins, ix, x, xi, 126
Proteins, biological value of, xi, 54, 257, 258, 259, 264, 265, 266, 267
Proteins, digestion of, x, xi
Proteins in nutrition, x, xi
Proteins, isolated, bacterial growth-stimulants in, 160
Proteins, maximum efficiency of, 55
Proteins, milk, 262 and ff
Proteins of cereal grains, relative values of, 55
Proteins, purified, nutritive value of, 6, 7, 8, 11, 12
Proteins, relative value in animal husbandry, 265
Protein utilization in pellagra, 246
Proteoses, 262
Prunes, content of *B* in, 82
Prunes, content of *C* in, 83
Pruritis, effect of yeast on, 311
Pseudo-betaine, 128
Psoriasis, effect of yeast on, 311
Pulses in antineuritic diet, 204
Pulses, thermostability of *B* in, 96
Purified protein as supplement to maize kernel, 49
Purine bases from rice, antineuritic properties of, 127
Purine bases, synthesis of in organism, 187
Purines, 126
Purines in the diet, 303
Putrefaction, abnormal intestinal, in beriberi, 173
Pyrimidine bases, synthesis of in organism, 187
Pyrimidines, 122, 127
Pyrimidines, antineuritic properties of, 127, 128, 182
Pyrimidines, hydroxy, antineuritic properties of, 127, 128

Quantitative determination of antiscorbutic vitamin, 33
Quantitative separation of *B* and *C*, 132
Quinine, antineuritic properties of, 127
Quinolinic acid, 128

Rabbits, feeding experiments with, 83, 234, 258 (footnote)
Rabbits, susceptibility to xerophthalmia of, 234
Rachitic diets, Mellanby's, 196, 197, 198
Radium emanation, effect on *B*, 98
Radium, similarity between effects of avitaminosis and, 183
Raspberry juice, thermostability of *C* in, 103
Rat, antiscorbutic requirements of, 166, 167
Rat feeding, methods, 22, 24
Rats, effect of deficiency of *A* on, 168, 169
Rats, experimental methods used with, 21 and ff
Rats, feeding experiments with, 3, 6, 7, 8, 9, 11, 12, 14, 15, 18, 19, 23, 24, 25, 27, 47, 48, 49, 50, 51, 53, 54, 55, 58, 64, 65, 72, 77, 80, 91, 93, 95, 98, 101, 113, 115, 117, 119, 120, 137, 142, 153, 158, 159, 160, 163, 166, 168, 169, 171 (footnote), 174, 175, 177, 179, 180, 210, 211, 236, 237, 238, 239
Rats, life cycle of, 19
Rats, polyneuritis in, 207
Rats, synthesis of *C* by, 166, 167, 221
Rats, value for experimental purposes, 19
Rats, vitamin requirements of, 165
Reconstructed milk, 297 (See also Enriched milk)
Reduction, effect on *A*, 94
Refining of oils, effect on vitamin content, 71, 113
Rennin, 263
Renovation of butter, effect on vitamin content of, 74
Reproduction, inadequacy of oat kernel for, 49
Reproduction, influence of mineral content on diet of, 48
Reproduction, influence of potato diet on, 53
Reproduction on diets deficient in *A*, 168

Reproduction on carotinoid-free diet, 115
Reproduction on dried milk diet, 270
Requirements of rats varying with age, vitamin, 175
Resistance, lowering of on vitamin-deficient diet, 172, 173, 237
Respiratory quotient, effect of *B* on, 187
Respiratory quotient in polyneuritis, 216 (footnote)
Rheumatic joints caused by lack of *C*, 179
Rhizopus nigricans, relation of vitamins to growth of, 315
Ribs, effect of lack of *A* on structure of, 99, 169, 170
Rice, biological value of protein of, 258
Rice bran, content of *B* in, 78
Rice, change in chemical composition due to polishing, 212, 213
Rice embryo, analysis of, 213 (footnote)
Rice embryo, separation of *B* from, 213 (footnote)
Rice germ, antineuritic value of, 82
Rice grain, antineuritic and growth-promoting power compared, 154
Rice grain, structure of, 212
Rice, "paddy," 212
Rice, "polished," 78, 212
Rice, polished, a factor in beriberi, 204 and ff
Rice, polished, nutritive deficiencies of, 15, 48, 213, 258
Rice, polished, starvation on diet of, 184
Rice polishings, antineuritic value of, 215
Rice polishings, effect of acid hydrolysis on, 118, 119
Rice polishings, effect of sodium hydroxide on *B* in, 119
Rice polishings, extract of antineuritic, 205
Rice polishings, extraction of *B* by alcohol from, 116
Rice polishings, isolation of *B* from, 121, 122, 125
Rice protein, amino acids of, 55
Rice starch in basal diets, 57
Rice, thermostability of *B* in, 95
Rice toxin, 15, 210, 211
Rice, unhusked, antineuritic value of, 150
Rice, unmilled, thermostability of *B* in, 94, 159
Rickets, 168, 170
Rickets, acidosis a factor in, 193, 194
Rickets, beneficial effects of exercise in, 196, 198
Rickets, calcium deficiency a factor in, 193, 195, 197, 199
Rickets, conditions predisposing to, 198, 199, 200
Rickets, deficient internal secretion, a factor in, 193
Rickets, domestication theory of, 195, 199, 200
Rickets, effect of phosphates and lime salts on, 64, 65
Rickets, etiology of, 193, 194, 195, 196, 199, 200
Rickets, fat deficiency a factor in, 194, 196 (footnote), 199
Rickets, geographic distribution of, 192
Rickets, heredity and, 193
Rickets, infection theory of, 193
Rickets, influence of phosphorus deficiency on, 65
Rickets, relation to breast-feeding, 193
Rickets, several types of, 199
Rickets, similar condition produced by phosphorus deficiency, 65
Rickets, social and economic factors in, 195
Rickets, symptoms of, 190 and ff
Rickets, treatment by Vitaphos, 148
Rickets, vitamin *A* as a factor in, 194 (footnote) 196 (footnote), 198, 199
Rind oil as preservative for orange and lemon juice, 103, 108 (footnote)
Rolled oats in the diet, 51, 52
Roots and tubers, variation in content of *A*, 75
Roots, colored, content of *A* in, 75
Roots, colorless, content of *A* in, 75
Roots, content of *A* in, 73
Roots, content of *C* in, 85
Roots, thermostability of *A* in, 92
Roughage, 22, 23, 103
Rutabaga, content of *B* in, 82
Rye, antineuritic value of, 215
Rye protein in the diet, 51, 52
Rye protein, nutritive value of, 55

Saccharomycetis as test for *B*, 35
Salivary glands, stimulation by vitamins, 184
Salt content of human and cow's milk compared, 268
Salted meat, antineuritic value of, 97
Salt mixtures, 59 and ff
Salts, mineral, ix, x
Sanctorius, 1
Sanitation a predominating factor in pellagra, 248, 249

Saponification, effect on extract of *A*, 112
Saponification of oils, effect on *A*, 112, 113
Saponified butter-fat, extraction of *A* from, 113
Scalded milk, antiscorbutic value of, 105
Sciatic nerve, degeneration in polyneuritis, 150
Sclerotinia cinerea, relation of vitamins to development of, 315
Scorbutic lesions, relation of *A* to, 228 (footnote)
Scurvy and beriberi, clinical link between, 218, 219, 220
Scurvy, bacterial origin of, 224, 225
Scurvy, chronic, poisoning a factor in, 223
Scurvy, constipation a factor in, 226, 227
Scurvy due to overcooking of vegetable food, a factor in, 306
Scurvy, etiology of, 223 and ff
Scurvy grass, 232
Scurvy, guinea-pig, symptoms of, 221, 222, 223
Scurvy in children on diet for tuberculosis, 232
Scurvy, infantile, symptoms of, 219, 220
Scurvy, latent, in children, 219, 220
Scurvy, relation between calcium metabolism and, 228
Scurvy, secondary factors in infantile, 285
Scurvy, susceptibility of different species to, 220, 221
Scurvy, symptoms of in adults, 217, 218, 219
Scurvy, treatment in 18th century, 229, 230
Scutellum of corn, *B* in, 78
Seal liver, antiscorbutic value of, 230
Seasonal variability of experimental animals, 31
Seasonal variation in vitamin-content of milk, 276, 277
Secretin, 185, 186
Seed and leaf mixtures, biological value of, 52
Seed and tuber mixtures, biological value of, 53
Seed, distribution of *B* within, 78, 79
Seed proteins, biological value of, 50, 51, 52, 53
Seeds, content of *A* in, 73, 74, 75
Seeds, content of *B* in, 77 and ff
Seeds, dry, content of *A* in, 77
Seeds, dry, content of *C* in, 83
Seeds, germinating, antiscorbutic value of, 83, 84
Seeds, inorganic salts in, 52
Seeds, thermostability of *B* in, 94, 95, 96
Selection of breeding stock, 28, 29
Separation of antineuritic and antiscorbutic factors, 132
Separation of antineuritic and growth-promoting factors, 153, 154, 159, 160
Separation of *B* from rice germ, method of Sawamura, 213 (footnote)
Septicaemia, relation between lack of vitamins and susceptibility to, 172
Seroglobulin, 264
Sexual organs, effect of lack of vitamins on, 174 (See also Testicles and Ovary)
Silica poisoning a cause of pellagra, 247
Silver skin, 204, 212
Size of seeds and content of *A*, relation between, 75
Skin, atrophic disorders due to lack of vitamins, 309
Social and economic factors in causation of rickets, 195
Soda biscuits, nutritive properties as compared with bread, 120
Soda, effect on *A* in oils, 90, 112
Soda, effect on *B* in navy beans, 96
Sodium bicarbonate, effect on *B* in cabbage, 120
Sodium bicarbonate, effect on *B* in navy beans, 120
Sodium hydroxide, effect on *B* in yeast, 120
Sodium hydroxide, effect on *C*, 133
Sodium hydroxide, effect on *B*, 119
Solubility of *A*, 111 and ff
Solubility of *B*, 115 and ff
Soy bean, content of *B* in, 77, 81
Soy beans, thermostability of *B* in, 98
Soy bean, wheat flour as supplement to, 77
Spermatogenesis, effect of lack of vitamins on, 174
Spinach, content of vitamin *A* in, 72, 73, 136, 137
Spinach, content of *B* in, 81
Spinach, content of *C* in, 85
Spinal cord, content of *B* in, 14, 152
Spinal cord, occurrence and function of vitamin in, 182
Spinal cord, condition of motor cells in polyneuritis, 183
Spinal cord, growth-promoting value of, 14
Spleen, effect of lack of *A* on, 169

Spleen, effect of lack of *B* on, 171, 173
Spleen, storage of vitamins in, 164
Spray process for drying of milk, 287, 291
Sprouted seeds, antiscorbutic value of, 83, 84, 232
Spruce beer, antiscorbutic value of, 84
Squash, thermostability of *A* in, 92
Stabilizer of vitamins, 129
Standardization of methods of vitamin research, 44
Standardization of vitamin dosage, 131
Starch as supplement to milk, 270, 271
Starch in basal diets, 57
Starch, relation between vitamins and, 173
Starvation on rice diet, 184
Stasis, intestinal, relation of vitamin deficiency to, 172, 311
Stasis, venous, relation to oedema of, 172
Steam, destruction of *A* in palm oil by, 92
Sterility, lack of vitamins a factor in, 174
Sterilization of foods without loss of antiscorbutic value, 103, 104
Sterilization of milk, 278, 279
Sterilized cereals, feeding experiments with fowls on, 208
Sterilized diet, effect on human beings of, 203, 204
Sterilized foods, content of *B* in, 98
Stimulants of gastric secretion, 6, 7
Stomach, effect of lack of *B* on, 171
Stomach, effect of lack of vitamins on, 171, 173
Storage, effect on antiscorbutic value of cabbage, 105, 106
Storage, effect on antiscorbutic value of carrots, 105
Storage, effect on antiscorbutic value of dandelion leaves, 105
Storage, effect on antiscorbutic value of foods, 105
Storage, effect on antiscorbutic value of potatoes, 105
Storage influence of on vitamin content of butter, 74
Storage of vitamins in animal body, 58, 164, 167, 168
Streptococci, growth-stimulant for, 160
String beans, variation in antiscorbutic value of, 84
Structure of vitamin *A*, 112
Structure of vitamin *B*, 128 (footnote), 129
Subcutaneous administration of vitamins, 184
Sugar an ingredient of experimental diets, 6, 8, 10, 14
Sugar beet, content of *A* in, 73
Sugar cane products a factor in pellagra, 247
Sugar in blood, predisposition to beriberi caused by excess of, 202
Sulphur a constituent of *B*, 132
Sulphur in lactalbumin, 263
Sulphur in protein-free milk, 268
Sulphuric acid, inactivation of *B* by, 118
Summary of physiological function of vitamins, 188
Sunlight a factor in onset of pellagra, 247
Sunlight, remedial effect in rickets, 195
Susceptibility of scorbutic animals to infection, 219
Susceptibility to infection, effect of lack of vitamins on, 172, 173
Susto, 149
Sweat glands, stimulation by vitamins, 184
Swedish turnip, content of *C* in, 85, 232
Swedish turnip juice, thermostability of *C* in, 101
Sweetwort, antiscorbutic value of, 84
Swine, feeding experiments with, 25, 47, 48, 70, 194 (footnote), 221, 265
Swine, nutritive requirements compared with those of rats, 20
Swine, polyneuritis in, 207
Swine, scurvy in, 221
Symptoms of beriberi, 206, 207
Symptoms of deficiency of *A*, 167 and ff
Symptoms of deficiency of *B*, 170 and ff
Symptoms of deficiency of *C*, 177, 178
Symptoms of guinea-pig scurvy, 221, 222, 223
Symptoms of pellagra, 243 and ff
Symptoms of polyneuritis in birds, 207
Symptoms of polyneuritis in cats, 208
Symptoms of polyneuritis in dogs, 208
Symptoms of rickets, 190 and ff
Symptoms of scurvy in adults, 217, 218, 219
Synthesis of vitamins, 77, 164, 166, 167, 316
Synthesis of *B* by bacteria, 160, 161
Synthesis of *B* by yeast, 43, 314, 315
Synthesis of *C* by chickens, 221
Synthesis of *C* by rats, 166, 221
Synthetic diets, 2, 6 and ff
Syphilis, relation of rickets to, 193

Tablets, vitamin, 149
Tadpoles, vitamin requirement of, 163
Technique of vitamin tests, 25, 33
Teeth, effect of vitamins on calcification of, 177, 178
Temperature conditions, regulation of in feeding experiments, 22, 23
Temperature, influence on destruction of *B* in cabbage, 99, 100
Temperature measurements, errors in, 89
Temperature, relation between rate of destructive of *C* and, 185
Testes, degeneration on vitamin-free diet, 162 (footnote), 169, 174
Testicles, effect of lack of *B* on, 171, 173, 174
Testicles, storage of vitamins in, 164
Tests for *B*, color, 131
Test for *C*, 135
Tethelin, antineuritic properties of, 128, 182
Tetrahydroxy pyridine, 128
Theory of vitamin action, 129, 130
Thermostability of *A*, 90, 91
Thermostability of antineuritic and growth-promoting factor compared, 154 and ff
Thermostability of *B*, 94 and ff
Thermostability of *B* in presence of alkalis, 119, 120
Thermostability of *C*, 133
Thermostability of *C* in absence of oxidizing agents, 133
Thermostability of *C* in milk, 284, 285
Thermostability of *C* in orange juice, 133
Thermostability of vitamin *C* in presence of acids, 134, 135
Thermostability of *C* in presence of alkalis, 134
Thymin, 123, 127
Thymus, content of *B* in, 82
Thymus, effect of lack of *B* on, 171, 173
Thymus, concentration of *B* from, 138, 139
Thymus, storage of vitamins in, 164
Thymus, vitamin content of, 182
Thyroid, desiccated, antineuritic properties of, 128
Thyroid, effect of lack of *B* on, 171, 173
Thyroid, effect of lack of *C* on, 177
Thyroid, concentration of *B* from, 139
Thyroxin, antineuritic properties of, 128, 182
Time curve of destruction of *C* in tomato juice, 102
Tikitiki extract, 235
Timothy, content of *A* in, 72, 73, 136, 137
Timothy, content of *B* in, 81
Timothy, dried, as source of *A*, 137
Tissues, effect of lack of *A* on, 167, 169
Tissues, effect of lack of *B* on, 171 and ff
Tissues, effect of lack of *C* on, 177
Tissues, muscular, effect of lack of vitamins on, 172
Tissues of polyneuritic birds as source of antineuritic, 167
Tomato, antiscorbutic value of, 83, 232
Tomato as antiscorbutic for infants, 298
Tomato, content of *B* in, 81
Tomato, cooked, antiscorbutic value of, 101
Tomato, dried, as source of *A*, 136
Tomatoes, canned, antineuritic value of, 101
Tomatoes, canned, antiscorbutic value of, 101
Tomatoes, canned, vitamin content of, 102
Tomatoes, content of *A* in, 74
Tomatoes, dried, as source of *C*, 108
Tomatoes, fresh, dosage for protection against scurvy, 101
Tomatoes, thermostability of *B* in, 96
Tomatoes, thermostability of *C* in, 101, 102
Tomato juice, canned, use in infant feeding, 102
Tomato juice, dried, antiscorbutic value of, 109
Tomato juice, thermostability of *C* influenced by hydrogenation concentration, 102 (footnote)
Tomato-seed, content of *A* in, 73 (footnote)
Tooth defects due to lack of vitamins, 178, 292
Tooth defects in scurvy, 217, 218, 222, 223
Tooth defects, relation of vitamins to, 309
Tooth-formation and vitamin supply, relation between 177, 178
Torulin, 122
Toxicity of common foodstuffs, 32
Toxicity of cotton seed, 32
Toxicity of magnesium, 32
Toxicity of peas, 50
Toxicity of rice, 15, 210, 211
Toxicity of wheat embryo, 48
Toxin a factor in pellagra, 258 (footnote)
Toxin, beriberi, 210, 211

Toxins in yeast test for *B*, 38
Trihydroxy pyridines, antineuritic properties of, 128
Triolein an ingredient of synthetic diets, 8
Tripalmitin an ingredient of synthetic diets, 8
Tristearin an ingredient of synthetic diets, 8
Trophic exchanges, regulation by vitamins, 162 (footnote)
Trumilk, 287
Tryptophane, xi, 54, 55, 258, 259
Tuberculosis a result of vitamin deficiency, 310
Tuberculosis, influence of lack of vitamins on susceptibility to, 172
Tuberculosis, pellagra in patients on diet for, 260
Tuberculosis, scurvy in children on diet for, 232
Tubers, amino acids in, 54
Tubers, and roots, variation in content of *A*, 75
Tubers and seed mixtures, biological value of, 53
Tubers, biological value of, 52, 53
Tubers, content of *A*, 73
Tubers, mineral content of, 52, 53
Tumors, relation of vitamins to, 181, 309 (footnote)
Tumors, vitamin content of, 182
Turnip, content of *B* in, 81, 82
Turnip, content of *C* in, 85, 221, 232
Turnip juice as source of *C* for infant feeding, 296, 298, 299
Turnip juice, swedish, thermostability of *C* in, 103
Turnip, swedish, thermostability of *C* in, 101
Turtle soup, antiscorbutic value of, 86
Typhoid bacillus, vitamin content of, 317
Tyrosine as supplement to lactalbumin, 267, 268

Ultraviolet rays, effect on *A*, 91
Unbalanced diet a factor in pellagra, 257
Unbalanced diets, vitamin requirement on, 257
University cream, 296
Unpalatability of synthetic diets, 7
Uracil, 123, 127
Urea in blood, effect of lack of *B* on, 174
Urea in milk, 262
Uric acid in milk, 262
Uric acid reagent, 131
Urethritis, effect of yeast on, 311
Urticaria, effect of yeast on, 311
Utilization of nitrogen, effect of lack of vitamins on, 180

Valine, xi, 125
Variability of *B* in tissues of the same type, 81, 82
Variability of experimental animals, 27, 28, 29, 30, 31
Variation in development of children, cause of, 299
Variation in nutritive value of vegetables with age, 101
Variation in vitamin requirements of individuals, 30, 31, 58
Variation of vitamin requirements with age, 175
Vasoconstriction, 173
Vasodilation, 174
Vasomotor disturbances, lack of vitamins a factor in, 174
Vegetable as compared with animal protein, biological value of, 258
Vegetable fats, antirachitic value of, 197, 198
Vegetable fats and oils, content of *A* in, 71
Vegetable foods as source of vitamins in the diet, 304, 305
Vegetable oils, 12
Vegetable oils, effect of hydrogenation on vitamin content of, 113
Vegetable oils, processed, investigations of, 114
Vegetables, antiscorbutic value of, 84, 85, 86
Vegetable soup as milk diluent in infant feeding, 296
Vegetables, scurvy developing on a diet containing, 86
Vegetables, variation in nutritive value with age, 101
Velvet bean, Georgia, content of *A* in, 73, 74 (footnote)
Velvet bean, Georgia, content of *B* in, 74 (footnote)
Velvet bean, Georgia, thermostability of *A* in, 92
Venous stasis, relation to oedema of, 172
Vitamin *A* in fats, distribution of, 66 and ff
Vitamin *A* in leaf, 52
Vitamin *A* in infant feeding, sources of, 296

Vitamin *A*, requirement of in human nutrition, 198
Vitamin *A*, structure of, 112
Vitamin *A*, synthesis by plants, 77
Vitamin action, theory of, 129, 130
Vitamin *B*, color tests for, 131
Vitamin *B*, discovery of, 15
Vitamin *B*, distribution within seed, 78, 79
Vitamin *B*, effect in infant feeding, 296
Vitamin *B* in foodstuffs, 77 and ff
Vitamin *B*, relative amounts in plant tissues, 80, 81
Vitamin *B*, symptoms of deficiency of, 170 and ff
Vitamin *B*, variability of in tissues of the same type, 81, 82
Vitamin-chocolate, 148
Vitamin content, correlation with function, 74, 75
Vitamin *D*, 159, 314
Vitamin *D*, separation from autolyzed yeast, 159, 160
Vitamin deficiency in children, symptoms of, 292
Vitamin hypothesis of Funk, 13, 16
Vitamin index, 80, 162 (footnote)
Vitamin, nomenclature, 3, 4, 13
Vitamin requirements for maintenance and growth, 162, 163
Vitamin requirements of adults, 300
Vitamin requirements of different species 165, 166
Vitamin requirements of pigeons, 165
Vitamin requirements of rats, 165
Vitamin requirements, variation in, 30, 31, 32
Vitamin research, criticism of, 34
Vitamin reserve in infants, 299
Vitamin tests, technique of, 25, 33
Vitamin theory, historical development of, 6
Vitamins, chemical structure of, 129
Vitamins, pharmacological action of, 129, 130
Vitamon, 149
Vitaphos, 147
Vitellin, 264

Walnut, content of *A* in, 74
War amenorrhea, 174
Water in diet, ix
Water, loss from the body, ix
Water, solubility of *A* in, 112
Water, solubility of *B* in, 115, 153
Water-soluble *C*, 16
Water-soluble vitamin, 4, 15, 16
Waxes extracted from plant leaves by solvents, 111
Waxes in plant extracts interfering in feeding experiments, 111
Weakness of women, effect of *B* on, 311
Wet beriberi, 206, 207
Whale oil, thermostability of *A* in, 90
Whale oil, vitamin content of, 68
Wheat, biological analysis of, 46, 47
Wheat bran, content of *B* in, 78, 82
Wheat, content of *B* in, 77, 215
Wheat embryo, antineuritic and growth-promoting power compared, 155
Wheat embryo, nutritive value of, 48
Wheat embryo, protein of, 48
Wheat embryo, thermostability of *B* in, 95
Wheat embryo, toxicity of, 48
Wheat endosperm, content of *B* in, 150
Wheat flour, white, deficiency in *B*, 78
Wheat germ, antineuritic value of, 77, 82, 215
Wheat germ, effect of sodium hydroxide on *B* in, 119
Wheat germ, extraction of *B* from, 117
Wheat, highly milled, a factor in etiology of beriberi, 204
Wheat, milk-producing value of, 265
Wheat, milling of, 214
Wheat, nutritive value of, 15
Wheat protein, biological value of, 47, 49, 51, 55
Wheat protein, relation of intake to growth, 48
Wheat, toxicity of, 32, 48
Whey, 263
Whey, effect of heat on, 280
Whey proteins, 262
Whey, thermostability of *B* in, 97
White flour, deficiency of, 214
White rice, milling of, 212
Whole wheat, toxicity of, 32

Xanthophyll, 112
Xanthophyll in pork liver, 77
Xerophthalmia, 168
Xerophthalmia, etiology of, 237, 238, 239
Xerophthalmia, factors influencing, 240, 241
Xerophthalmia in calves, 165, 167
Xerophthalmia in children, 235, 236
Xerophthalmia in rats, 31, 168, 169, 237, and ff
Xerophthalmia, pathology of, 234

Xerophthalmia, resistance of mature animals to, 240
Xerophthalmia, species susceptible to, 234, 235
Xerophthalmia, symptoms of, 234, 235
Xerophthalmia, treatment for, 235, 236
Xerophthalmia, two forms of, 238
X rays, similarity between effects of avitaminosis and, 183

Yeast, antineuritic value of, 14, 77, 215, 216, 305
Yeast, antineuritic and growth-promoting power compared, 152
Yeast, antirachitic value of, 196
Yeast as addition to food, 148, 305
Yeast as cure for xerophthalmia, 238, 239
Yeast, autolyzed, antineuritic value of, 77
Yeast, autolyzed, bacterial growth-stimulant in, 160
Yeast, autolyzed, effect of drying on vitamin from, 96
Yeast compared with milk as source of *B*, 269
Yeast compared with protein-free milk as supplement to diet, 266
Yeast, dried, as source of *B*, 81
Yeast, effect of alkali on *B* in, 120
Yeast, effect of radium emanation on *B* in, 98
Yeast, growth-promoting factor in, 152 153
Yeast, isolation of *B* from, 122, 123, 125, 126, 127
Yeast, pressed, content of *B* in, 77, 82
Yeast, process for increasing edibility of, 143
Yeast, proliferation of, 313 (footnote)
Yeast stimulant as distinct from *B*, 159, 160
Yeast, stimulant for invertase activity of, 160 (footnote)
Yeast, stimulation by vitamins, 314, 315
Yeast test for *B*, 35 and ff, 120, 151, 159
Yeast test for *B*, comparison with rat tests, 39, 41, 42
Yeast test for *B*, Vitamin *C* a confusing factor in, 39
Yeast, thermostability of *B* in, 96
Yeast, vitamin requirements of aerobic and anaerobic varieties compared, 187

Zein, 258
Zein, bacterial growth-stimulant in, 160
Zone distribution of pellagra, 248, 249